PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE LEAVES OF *Citrullus lanatus* (Thunb.) Matsum. & Nakai. (CUCURBITACEAE)

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University
Chennai-600 032

In partial fulfilment of the requirements
for the award of the degree of

MASTER OF PHARMACY
IN
PHARMACOGNOSY

Submitted by

261220709

DEPARTMENT OF PHARMACOGNOSY

COLLEGE OF PHARMACY
MADURAI MEDICAL COLLEGE
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APRIL 2014

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CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE LEAVES OF *Citrullus lanatus* (Thunb.) Matsum. & Nakai, (CUCURBITACEAE)''** submitted by **Miss. K.VIJAYALAKSHMI (Reg. No. 261220709)** in partial fulfilment of the requirements for the award of the degree of **MASTER OF PHARMACY** in **PHARMACOGNOSY** by The Tamil Nadu Dr. M.G.R. Medical University is a bonafied work done by her during the academic year 2013-2014 under the guidance of **Dr.(Mrs). AJITHADAS ARUNA, M.Pharm., Ph.D.,** Joint Director of Medical Education (Pharmacy), in the Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai-625 020.

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CERTIFICATE

This is to certify that the specimen brought by **Miss. K.VIJAYALAKSHMI,** II.M. Pharmacy, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai is identified as ***Citrullus lanatus (Thunb.) Matsum. & Nakai,*** belonging to the family **Cucurbitaceae**.

STATION : Madurai.

DATE :

(Dr. D. STEPHEN)

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ACKNOWLEDGEMENTS

I first and foremost express my revered regard and obeisance to the **ALMIGHTY GOD** with whose blessings I was able to complete my project work.

I am grateful to express my sincere thanks to **Dr. B. SANTHAKUMAR, M.Sc (F.Sc)., M.D(F.M)., PGDMLE, Dip.N.B (F.M).,** Dean, Madurai Medical College for giving me an opportunity to carry out my project work.

I sincerely thanks with heartfelt sense of gratitude to **Dr. L. SANTHANALAKSHMI, M.D., D.G.O., M.B.A.,** Vice Principal, Madurai Medical College for giving me an opportunity to carry out my project work.

It is my privilege to express a deep and heartfelt sense of gratitude and my regards to our respected **Dr. Mrs. AJITHADAS ARUNA, M. Pharm., Ph.D.,** Joint Director of Medical Education (Pharmacy) and former Principal, College of Pharmacy, Madurai Medical College and for her active guidance, advice, help, support and encouragement. I am very much obliged for her perseverance, without which my project work would not be completed.

I owe a great debt of gratitude and heartful thanks to **Dr. A. ABDUL HASAN SATHALI, M. Pharm., Ph.D.,** Principal (in charge) and Head of Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai

I express my heartful thanks and regards to **Miss R. GOWRI, M.Pharm., Dr.K.PERIYANAYAGAM,M.Pharm.,Ph.D.,** and **Mr.T.VENKATARATHINA KUMAR,M.Pharm.,(Ph.D).,** Assistant Readers in Pharmacy, Department of

Pharmacognosy, College of Pharmacy, Madurai Medical College for their support and valuable suggestions.

I thank **Prof. Mrs. R. THARABAI, M. Pharm,** Professor and Head of Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College for their guidance during the course of my study.

I thank **Mrs. A. SETHURAMANI, M.Pharm.,(Ph.D).,** and **Dr.Mrs.A. KRISHNAVENI, M. Pharm., Ph.D.,** Tutors in Pharmacy, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College for their help.

I thank **Mr. P. SIVAKUMAR M.Sc., DMLT,** Lab Supervisor of the Dept. of Pharmacognosy and **Mr. MAGUDESWARAN, DMLT,** Lab Technician and **Mrs. P. ELLAYEE** for their support during my study of this work.

I extend my thanks to **all the teaching staff** of College of Pharmacy and of Madurai Medical College who have rendered their help towards completion of the project.

I place on record my gratitude to **Dr. P. JAYARAMAN, M.Sc., Ph.D.,** Director, Plant Anatomy Research Centre, Chennai 600 045 who helped me in the microscopic studies and **Dr. D. STEPHEN, Ph.D.,** Senior Lecturer, Department of Botany, American College, Madurai who helped me in the identification of my plant.

I thank **Dr. Mr. MARIYAPPAN (Scientist)**, Indian Council of Medical Research, Madurai for his kind guidance for study of my project work.

I am thankful to **Mr. Jones** Universal Scientific Supplier for his timely supply of chemicals utilized during the project work.

I express my gratitude to **Mrs. Anithakumari** of AVN formulations Madurai for her support and help in carry out HPTLC analysis of this work.

I also thank my ever loving classmates, **Miss. P. Anitha, Miss. P. Bala, Miis. R. Jancy Gracelet, Mr. S. Jegadeesh, Miss. M.Kalaiyarasi, Mrs. S.R.Nandhini, Mrs. S.Nathiya, Miss. D.Suganya, Mr.A.Manikkavasagan, Mr.J.Rajesh Kumar, Mr.Sankar Ganesh, Mr.S.Sudhakar, Mr.M.Ramanathan, Mr.P.Kanniyappan, Mr.P.Arjun Kumar, Mrs.S.Ponnammal Asmi, Mr.C.Pravin Kumar, Miss.R.Elavarasi, Miss.S.Karpagam, Miss.E.Ajila, Mr.K.Sasikumar** and all my juniors of 2013-2015 batch and other friends for their constant motivation and help.

Above all, I am forever indebted to my father **Mr. M.KANNAPPAN,** my mother **Mrs. K. PUSHPAM,** my brother **Mr. K. MANIKANDAN** and **Mr. P. MOHAN** for their understanding, endless patience, help and encouragement which made me to complete this work.

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INTRODUCTION

CHAPTER I

INTRODUCTION

Medicinal Plants [1-3]

A plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for semi-synthetic compounds. When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes.

History of medicinal plants

Plants have been used for medicinal purposes from 5000 BC with the emergence of the Indus Valley Civilization. The indigenous system of medicine, viz.-Ayurvedic, Siddha and Unani, have been in existence for several centuries. The country has 45,000 different plant species and 15000 medicinal plants that include 2000 plants used in Ayurveda, 700 in Unani, 600 in Siddha, 450 in Homoeopathy and 30 in modern medicines. The drugs are derived either from the whole plant or from different parts like leaves, stem, bark, root, flower, seed etc. Some drugs are prepared from excretory plant product such as gum, resins and latex.

Significance of medicinal plants to human beings

(1) Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.

(2) Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.

(3) Many food crops have medicinal effects, for example garlic.

(4) Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species.

Hence studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants.

Future of medicinal plants

Medicinal plants have a promising future because there are about half million plants around the world, and most of their medical activities have not been investigated yet, and their pharmacological activities could be decisive in the treatment of present or future studies.

Characteristics of medicinal plants

Medicinal plants have many characteristics when used as a treatment, as follow:

- **Synergic medicine** - The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.
- **Support of official medicine** - The components of the plants proved to be very effective in the treatment of complex cases like cancer diseases.
- **Preventive medicine** - It has been proven that the component of the plants also has the ability to prevent the appearance of some diseases which can help to reduce the use of the chemical remedies and reduce the side effect of synthetic treatment.

Medicinal plants in India

About 60 percent of the world's population use herbal medicines. Herbal medicines are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used.

There are about 45,000 medicinal plant species in India, concentrated in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000 plants. India is the largest producer of medicinal herbs and is called the botanical garden of the world.

Ayurveda and Kabiraji (herbal medicine) are two important forms of alternative medicine that is widely available in India. Ayurveda form of medicine is believed to be existent in India for thousands of years.

The codified traditions have about 25,000 plant drug formulations that have emerged from such studies. In addition to this, over 50,000 formulations are believed to be available in the folk and tribal traditions. All these point to the deep passion for an exhaustive knowledge about medicinal plants that have existed in this land from time immemorial.

Importance of Medicinal Plants [4]

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs.

Integrating the use of Traditional medicine (TM) in the treatment of incurable disease such as AIDS to boost immunity is wiser than waiting for the immune system to weaken to begin antiviral therapy as is the common practice, especially when evidence exists that 11 of the anti infective herbs in Chinese TM have shown to be anti-HIV.

According to the WHO, 25% of modern medicines are made from plants first used traditionally. One recent example is the use of Artemisinin based drugs for treating malaria due to the malaria parasite exhibiting drug resistance to previously prescribed drug therapies. Traditional Chinese medicine has been used to effectively treat malaria with cultivated *Artemisia* plants for over 2500 years.

In South Africa, the medical research council is conducting studies on the efficacy of the plant *Sutherlandia microphylla* in treating AIDS patients. Traditionally used as a tonic, this plant may increase energy, appetite and body mass in people living with HIV.

Diabetes mellitus is another area where a lot of research is going on. *Ajuga reptens* (the active principle is said to potentiate effects of insulin), *Galagea officinalis* (galagine), *Bougainvillea spectabilis* (pinitol), *Momordica charantia* (chirantin), *Gymnema sylvestre* (gymnemic acid) are some medicinal herbs that have shown effectiveness in non-insulin dependent diabetes. Recently extract of *Tecoma stans* has shown potent anti diabetic activity. Alkaloid tecomonine is considered to be active principle of the herb.

Arthritis is another potential disease where no satisfactory answer is present in modern medicine. *Commiphora mukul* (guggulsterones), *Boswellia serrata* (boswellic acid), *Withania somnifera* (withanolides), *Ruscus acueleatus* (ruscogenin) are prominent plants with anti-arthritic activity.

Croton sublyratus (plaunoyol) has potent and wide spectrum anti peptic ulcer action. *Ancistrocladus korupensis* (michellammine-b), *Caulophyllum langigerum* (calanolide-A), *Caulophyllum teymani* (costatolide-A), *Homalanthus natans* (prostratin) are the medicinal herbs from African countries that are being employed in research for finding a suitable cure for Aids.

Some Common Major Diseases [5]

Some common major disease described by the Dirnasa tribe are jaundice, diabetes, high blood pressure, urinary tract infection, carbuncles, cardiac problem, cancer, hearnaturia. Malaria, filariasis, Japanese encephalitis, dengue hemorrhagic fever, chikungunya and yellow fever are transmitted by mosquitoes which cause millions of deaths every year.

Modern medicine discovered from plants [6]

Plants provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine among others. In some cases, the crude extract of medicinal plants may be used as medicaments. About 121 (45 tropical and 76 subtropical) major plant drugs have been identified for which no synthetic one is currently available.

It has been estimated that more than 400 traditional plants or plant-derived products have been used for the management of type 2 diabetes across geographically. Galegine, a substance produced by the herb *Galega officinalis*, provides an excellent example of such a discovery. Experimental and clinical evaluations of galegine, provided the pharmacological and chemical basis for the discovery of metformin which is the foundation therapy for type 2 diabetes. Plant derived agents are also being used for the treatment of cancer. Several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodophyllotoxin are in clinical use all over the world.

The use of herbal products is of global importance because of their low side effects, accessibility and affordability when compared with conventional medicine.

Advantages of Herbal Medicines compare with alternative therapy [7-8]

Herbal medicine has been used for centuries to treat many different health conditions. As with most types of complementary or alternative therapy, people may use it to help themselves feel better or feel more in control of their situation. Herbal medicine is often promoted as a natural way to help you relax and cope with anxiety, depression and other conditions such as hay fever, irritable bowel syndrome, menstrual (period) problems and skin conditions such as eczema.

In India it is proving to be a major health problem, especially in the urban areas. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects, low cost, widely available and less toxic compared with allopathic drugs.

***CITRULLUS LANATUS* (Watermelon)** [9]

Citrallus lanatus (water melon) produces a fruit that is about 93% water, hence the name "water" melon. The "melon" part came from the fact that the fruit is large and round and has a sweet, pulpy flesh. The scientific name of the watermelon is derived from both Greek and Latin roots. The *Citrullus* part comes from a Greek word "citrus" which is a reference to the fruit. The *lanatus* part is Latin, and has the meaning of being wooly, referring to the small hairs on the stems and leaves of the plant (Baker, *et al*., 2012). Watermelon is thought to have originated in southern Africa because it is found growing wild throughout the area, and reaches maximum diversity of forms there. It has been cultivated in Africa for over 4,000 years. *Citrullus lanatus* was brought to America by Spanish and quickly became very popular crop (Robinson and Decker, 1997).

- *Citrullus lanatus* (Water melon) has been reportedly used widely in traditional herbal medicine. The **fruit** is used as a febrifuge, diuretic, purgative and used in treatment of diarrhoea, gonorrhoea, dropsy and renal stones.
- The **leaves** of *Citrullus lanatus is* analgesic, anti-inflammatory, mosquitocidal, gonorrhoea and anti microbial property.
- The **fruit** is also diuretic, anti-cancer, high BP, antiviral and is effective in the treatment of dropsy and renal stones. The seed is also a good vermifuge and has a hypotensive action. Preliminary research indicates that the consumption of watermelon may have antihypertensive effects.
- The **root** is purgative and in high dose it can also serve as emetic.
- The **seed** is a good vermifuge and has a hypotensive action. It is a demulcent and used in the treatment of the urinary tract infections as well as bed wetting.
- **Fatty oil in the seed**, as well as aqueous or alcoholic extracts, had been reported to paralyze tapeworms and roundworms.
- The **rind of the fruit** is prescribed in cases of alcoholic poisoning and

The plant *Citrullus lanatus* has been selected (specially the leaves) for the present investigation on the basis of the ethnomedical information and the review of literature as the plant is widely cultivated throughout India.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

A literature review is an evaluative report of studies found in the literature related to the selected area of research. The review describes, summarizes, evaluates and clarifies the literature available in the present research. It is a step towards further investigation on a particular work. It denotes with works derived from primary and secondary sources.

Mallavarapu GR and Rao LR (1979) isolated the chemical constituents of some Cucurbitaceae plants including *Citrullus colocynthis*. Cucurbitacin B,E and I and Cucurbiracin-E-2-glycoside were isolated from *Citrullus colocynthis*.[10]

Tripathi SN *et al*., (1980) evaluated the hypoglycemic activity of certain indigenous drugs including *Citrullus colocynthis* in rabbits. The response of these drugs on glucose induced hyperglycaemia. [11]

Itoh T *et al*., (1981) has demonstrated the co-occurrence of the C-24 epimers spinasterol and chondrillasterol in seeds of *Citrullus lanatus* (Cucurbitaceae) and seeds of bottle guard (*Langenaria leucantha* var. *gourda*) by ^{13}C NMR spectroscopy method.[12]

Yohora SB and Khan MSY (1981) studied the diuretic activity of *Albizzia lebbeck* (seeds saponin), *A. odoratissima* (seeds, saponin), *Annona squamosa* (seeds), *Cicer arietinum* (seed coat), *Citrullus colocynthis* (seeds), *Lepidium sativum* (seeds), *Nigella sativa* (seed, seed oil), *Ochrocarpus longifolius* (flowers, flavonoids), *Peucedanum grande* (seed, oil), *Solanum xanthocarpum* (seeds, saponin), *Taxus baccata* (leaves) and *vitex nigando* in rats when compared to that of urea. The seed coat of *Cicer arietinum* and *T. baccata* exhibited maximum diuretic activity.[13]

Hussein Ayoub SM and Yankov LK (1981) isolated 8 components from peels of *Citrullus colocynthis* (Cucurbitaceae) by column chromatography and preparative TLC. The less polar component represented an alkaline mixture (mp 38-42°C).[14]

Hussein Ayoub SM and Yankov LK (1981) examined the free hydroxyl and carbonyl components mixture from the petroleum ether fraction of *Citrullus colocynthis* peels by GLC.[15]

Nag TN and Harsh ML (1982) reported the presence of steroidal sapogenins viz., diosgenin, tryptogenin, lanosterol and beta sitosterol from various parts of *Citrullus colocynthis* (Cucurbitaceae).[16]

Hussein ASM and Yankov LK (1983) isolated the two isomers of 11,14 dimethyl hexadecane -14-ol-2-one ($C_{18}H_{36}O_8$) from petroleum ether extract of *Citrullus colocynthis* fruit peels (Cucurbitaceae).[17]

Harsh ML *et al*., (1983) studied the antimicrobial activity of petroleum ether and 50% ethanolic extract of roots, shoot and fruits of *Citrullus colocynthis* against *Staphylococcus aureus, E. coli* and *Candida albicans.* The extracts were found to be effective against the tested organisms. [18]

Pandey P *et al*., (1985) evaluated the effect of livol (R) a formulation on some biochemical parameters in relation to improvement of liver function. The composition of herbal drug include *Citrullus colocynthis* administered orally exhibited protective hepatotoxic activity in experimental dogs.[19]

Garg VK and Nes WR (1986) studied the sterol composition of 13 components of 6 Cucurbitaceae seeds including *Citrullus lanatus*. They were codisterol, 25 (27)-dihydroporiferasterol, clerosterol, isofucosterol, stigmasterol, campesterol, 22 (27)-dehydrochondrillasterol, 24-β-ethyl-25(27)-dehydrolathosterol, avenasterol, spinasterol, 24

epsilon methyl lathosterol and 22-dihydrospinasterol, 24-methylene cholesterol (small quantities). δ-5-sterol were noticed in all the species.[20].

Sushil Kumar *et al*., (1997) have reported the antibacterial activity of seeds 40 plant species including *Citrullus vulgaris* (Cucurbitaceae). The antibacterial activities of the seeds of 36 plant species were tested against *Pseudomonas cichorii, Bacillus substilis, Salmonella typhimurium* and *E. coli*. *Citrullus vulgaris* showed larger inhibition zones than the other tested species.[21]

Ziyyat A *et al*., (1997) studied antidiabetic activity of 41 plants including *Citrullus colocynthis* (Cucurbitaceae). The most used plants included *Trigonella foerumgraecum, Globularia alypum, Artemisia herbaalba, Citrullus colocynthis* and *Terraclinis articulate*. In the hypertension's therapy 18 vegetal species were reported, of which the most used were *Allium sativum, Olea europea, Arbutus unedo, Urtica dioica* and *petroselinum crispum*. Among the 18 species used for hypertension, 14 were also employed for diabetes. Moreover these two diseases were associated in 41% of hypertensives. These findings suggest that hypertension observed in this region would be in a large part related to diabetes.[22]

Rizvi MA *et al*., (1998) discussed the medicinal uses of some poisonous plants including *Citrullus colocynthis* (Cucurbitaceae) and also discussed botanical description, distribution, chemical and poisonous constituents.[23]

ESJ Nidiry (1998) studied the antifungal activity of various extracts of seeds of *Citrullus lanatus*. The methanolic extract exhibited higher activity against mycelial growth of *Collectotrichum gloeosporiodes* while the petroleum ether extract had higher activity against spore germination of *Cladosporium cucurmerinum*.[24]

Bhujbal MM (1999) studied the management of 50 cases of various skin diseases. They were treated with decoction mixture of *Trichosanthus*, *Citrullus colocynthis*, *Gentiana*

kurroo, Terminalia chebula and *Zingiber officinale* at a dose of 20-40mL on empty stomach with hot water and honey for 4-6 weeks. It was useful and no side effects were observed.[25]

Billore KV *et al*., (2000) studied the ethnobotanical lores in birth control of some species including *Citrullus* species, practiced traditionally by the tribals of Rajasthan in western India. The study may play a vital role in the prospective national birth control programme of the country.[26]

Ayangarya VS (2000) studied the treatment of all type of skin disease cured by the fruit juice of *Citrullus colocynthis*. The boils on the skin have been cured by applying the fruit juice on the body. A lotion was prepared from the fruits of *cucumis* and skin care benefits were also felt by the urbanites. Even, powder can be prepared from the fruits of this plant for the treatment during unseasonal days.[27]

Anuradha V *et al*., (2000) examined the highest larvae mortality of petroleum ether and benzene extract of 6 plants including *Citrullus colocynthis*. Highest mortality was observed in seed extract of *Citrullus colocynthis*. The percentage of adult emergene was 8.3. Extended larval periods, low fecuding and 100% mortality of second generation larvae were also observed.[28]

Al-yahya MA (2000) studied the toxicity of 10% of *Citrullus colocynthis* fruits or 10% of *Nerium oleander* leaves or their 1:1 mixture in rats. Dullness, ruffled hair, decreased body weight gains and feed efficiency and entero hepatoneuropathy characterized treatment with *Citrullus colocynthis* and *Nerium oleander* given alone. Diarrhea was a prominent sign of *Citrullus colocynthis*. Feeding the mixture of above two drugs caused more effect and death of rats.[29]

Shantha *et al*., (2001) have studied the pharmacognostic features of the seeds of *Citrullus lanatus*. They have described macroscopy, microscopy, histochemical tests,

solubility, physical constants, extractive values, and test for inorganic and organic constituents, UV and TLC studies of the seeds of *Citrullus lanatus*.[30]

Pino *et al*., (2003) have isolated the volatile oil components of *Citrullus lanatus* fruit by simultaneous steam distillation/solvent extraction method. The fruit had 7.6mg/kg of total volatile compounds.[31]

Bendjeddou D *et al*., (2003) studied the immuno stimulating activity of the hot water soluble polysaccharide extract of 3 plants including *Citrullus colocynthis*. The extract of *Citrullus colocynthis* showed weaker immune stimulating activity to *Anacyclus pyrethrum* and *Alpinia galanga* which showed a marked stimulating effect on the reticulo endothelial system.[32]

Paudel RC *et al.,* (2003) reported the anti-hepatitis activity of 44 plant species included *Citrullus colocynthis* and also tested the antiviral activity of this plant in China and India.[33]

Mukherjee A *et al*., (2003) evaluated the hepatoprotective activity of *Citrullus colocynthis* root against carbon tetrachloride induced toxicity in albino rats. Hepato-protective activity of different extracts of *Citrullus colocynthis* L. Sch. (roots) (Cucurbitaceae) was investigated in albino rats by inducing hepatotoxicity with carbon tetrachloride. The alcoholic extract of *Citrullus colocynthis* Sch. 100 mg/kg b.w. has been shown to posses significant hepatoprotective effect by lowering the serum level of transaminases (GPT and GOT), alkaline phosphate (ALP) and bilirubin ($P < 0.05$ to $P < 0.001$).[34]

Goswami DN (2003) examined the fatty acid composition of seeds of *Citrullus colocynthis* by various chromatographic and spectral technique.[35]

Fukushige H and Hilderbrand DF (2005) compared the highest hydroperoxide lyase enzyme activity of *Citrullus lanatus* (Cucurbitaceae) leaves with *Nicotiana tobaccum*

(Solanaceae) in transgenic leaves (50 times higher than endogenous HL activity). The enzyme is 3 times more active with 13-hydroperoxylinolenic acid than with 13-hydroperoxylinoleic acid. The activity against 9-hydroperoxides of polyunsaturated fatty acid is minimal when compared with Arabidopsis HL also expressed in N.tobaccum the highest HL activity is 10 time higher in watermelon.[36]

Kozan E *et al*., (2006) have evaluated the *in vivo* anthelmintic activity of ethanolic and aqueous extract of 9 plant species including *Citrullus lanatus* against pinworm, *Syphacia obvelata and Aspiculiris tetraptera* in mice. The ethanolic and aqueous extracts of *Citrullus lanatus* showed anthelmintic activity.[37]

Perkin S *et al*., (2006) have determined the carotenoid content (84.97%) of *Citrullus lanatus* by HPLC method and lycopene content of *Citrullus lanatus* by colorimetric assay. The total lycopene content was used to separate watermelon cultivars into low (more than 50mg/kg fw), average (50-70mg/kg fw), high (70-90mg/kg fw), and very high (less than 90mg/kg fw). Cultivars varied greatly in lycopene content, ranging from 33 to 100mg/kg).[38]

Jabbar A *et al*., (2006) reported the inventory of the ethnobotanical used as anthelmintics in southern Punjab (Pakistan). 3 stage process was used to document the plants being used to treat and/or helminthosis in ruminants. The main plants used were *Lamium amplexicaule, Mallotus philippinensis, Withania somnifera, Azadirachta indica* and *Citrullus colocynthis*. The study provided a foundation for the scientific study and verification of those plants used as anthelmintics.[39]

Nayab D *et al*., (2006) isolated the cucurbitacin glycoside from *Citrullus colocynthis* (Cucurbitaceae). A new cucurbitacin glucoside 2-O-β-D-glucopyranosyl-16α-20*R*-dihydroxy-cucurbita-1,5,23*E*,25(26)-tetraen-3,11,22-trione (**1**) has been isolated from the methanolic extract of the fruits of *Citrullus colocynthis*. The structure has been assigned on the basis of spectral analysis including 1D and 2D NMR techniques. In addition 2-O-β-D-

glucopyranosyl-cucurbitacin B (arvenin I) (**2**) and 2,25-di-O-β-D-glucopyranosyl-cucurbitacin L (**3**) are reported for the first time from this species.[40]

Sharma M and Vats S (2007) collected the ethnobotanical survey of digestive disorder cure plants. 16 species oncluding *Citrullus colocynthis* (Cucurbitaceae) were used by the tribal people of Rajasthan for curing digestive disorders.[41]

Qureshi R and Bhatti GR (2007) studied the *Citrullus colocynthis* medicinal properties used in traditional system of medicine i.e. Unani, Ayurvedic and Homeopathic. Its purgative action was due to the presence of alkaloids. This plant is commonly used for digestive complaints in human beings and livestock by traditional users in Nara desert, Pakistan. The medico ethno-botanical survey presented describing phytochemistry, medicinal properties, description and distribution of plants.[42]

Khowri NA *et al*., (2007) studied the effect of aqueous extract of *Citrullus colocynthis* (Cucurbitaceae) leaves on the lipid profile and other biochemical parameters on Albino rats. The extract showed decreases in total serum cholesterol level and decrease in blood level of both serum alanine and serum creatine kinase ($p \leq 0.05$) and increase in serum lactate dehydrogenase ($p \leq 0.01$) by oral administration of the extract to albino rats at a dose 500mg/kg for 7 days.[43]

Joshua AJ *et al*., (2007) examined the lipotropic activity of Natchol, a polyhedral formulation containing *Solanum nigrum* (Solanaceae), *Citrullus colocynthis* (Scrophulariacea), *Sida cardifolia* (Malvaceae) and *Boerhaavia diffusa* (Nyctaginaceae). The formulation was administered orally at 100 mg/kg b.w. while choline chloride was given at the dose of 200mg/kg b.w for 7 days prior to carbon tetrachloride treatment in albino wistar rats. Natchol treatment significantly reduced the hepatic triglyceride level and reduction in weight gain induced by carbon tetrachloride. The histopathological examination further confirmed the lipotropic activity.[44]

Aburjai T *et al*., (2007) studied the ethno-pharmacological survey of medicinal herbs including *Citrullus colocynthis* in Jordan, the Ajloun height region. The use of moderately unsafe or toxic plants by traditional healers was noted. These plants include *Ecballium elaterium, Euphorbia hierosolymitana, Mandragora autumnalis* and *Citrullus colocynthis*. Kidney problems scored the highest informant concensus factor (ICF) while *Cracus heymalis* was the plant of highest use value.[45]

Mukherjee A *et al*., (2007) evaluated the activity of alcoholic aqueous extract of roots of *Citrullus colocynthis* against carbon tetrachloride induced hepatotoxicity in albino rats. The extract showed a significant hepatoprotective activity.[46]

Yoshikava M *et al*., (2007) isolated the two new Cucurbitane type triterpene glycoside colocynthoside A and B with 17 known constituents and Cucurbitacin E2-O-β-D-glucopyranoside from methanolic extract of *Citrullus colocynthis* (Cucurbitaceae). The Cucurbitane type triterpene glycoside, Cucurbitacin E2-O-β–D-glucopyranoside and its aglycone, Cucurbitacin E exhibited antiallergic activity at 100 and 1.25mg/kg, p.o., respectively.[47]

Meena MC and Parni V (2008) isolated and identified the flavonoid "quercetin" from various solvent extractions of leaf, stem, fruit and root of *Citrullus colocynthis* (Cucurbitaceae). The purified material was subjected to IR, HPLC and identified as quercetin. The Rf value of isolated quercetin and standard quercetin was compared.[48]

Sunil Kumar *et al*., (2008) evaluated the phytochemical screening of methanolic extract of fruits of *Citrullus colocynthis* (Cucurbitaceae). The plant showed higher amount of phenolic and flavonoids. The phenolic content was 0.74% (calculated at gallic acid) and 0.13% of flavonoid calculated as catechin equivalents per 100g of fresh mass and antioxidant activity was evaluated and free radical scavenging effect also evaluated. The IC_{50} of the extract was found to be 2500µg/mL.[49]

Allali H *et al*., (2008) studied the most useful hypoglycemic activity of more than 58 plants including *Citrullus colocynthis* (Cucurbitaceae). The results gathered from 634 injury forms (435 women and 199 men) were separated into two groups; diabetic using medicinal plants (62%) and industrial hypoglycemic medicines (38%). The finding also showed non-insulin dependent patient used more medicinal plants than insulin dependent patients.[50]

Benmehdi H *et al*., (2008) studied the hypo and anti hyperglycemic effect of the seeds of *Citrullus colocynthis*. Intra peritoneal administration of the aqueous extract 1.25g/kg to *streptozotocin* induced diabetic rats produced reduction of blood sugar level in long term while the same extract produced no alteration of glycaemia in normal rats in short term. The extract has maximal adverse effect and high LD 100 value.[51]

Sangameswaran B *et al.*, (2008) studied the oral hypoglycemic activity of both aqueous and methanolic extracts of leaves of *Citrullus colocynthis* (Cucurbitaceae) in experimental animal (dose 500mg/kg). The standard anti diabetic agent, *glibenglamide* (500mg/kg) used to compare in this activity. The results indicate significant anti diabetic activity by both the test extracts ($p \leq 0.01$).[52]

Dineshkumar B *et al*., (2009) reviewed the anti diabetic activity of common Indian plants including *Citrullus colocynthis*. These may act on beta cells of the pancreas and stimulate the secretion of insulin, inhibits α-cells for the release of hypoglycemic factors, enhance the effect insulin, inhibit the synthesis of glucose 6-phosphate phosphatase, fructose diphosphatase, pyruvate carboxylase of phosphoenol pyruvate carboxykinase and stimulate the synthesis of glucokinase.[53]

Dhanotia R *et al*., (2011) evaluated the effect of *Citrullus colocynthis* Schrad fruits for hair growth in androgen induced alopecia. The petroleum ether extract was applied topically. Alopecia was induced in albino mice by simultaneous administration of extract were evaluated using follicular density, anagen/telogen (A/T) ratio and microscopic

observation of skin section. Petroleum ether extract of (*Citrullus colocynthis*) exhibited promising hair growth promoting activity, as reflected from follicular density, A/T ratio and skin sections. The treatment was also successful in bringing a greater number of hair follicles in anagenic phase than the standard Finasteride. The result of treatment with 2 and 5% petroleum ether extracts were comparable to the positive control Finasteride.[54]

Upadhyay B *et al*., (2011) reported the ethno-veterinary uses and informants consensus factor of medicinal plants of Sariska region, Rajasthan, India. The highest ICF (0.61) was scored for digestive problem. *Citrullus colocynthis* used for fever and general sickness with a highest use value (UV) of 0.62.[55]

AIM AND SCOPE

CHAPTER III

AIM AND SCOPE OF THE PRESENT STUDY

The use of herbal products is global importance because of their low side effects, accessibility and affordability when compared with conventional medicine. *Citrallus lanatus* (watermelon) is popular in indigenous system of folk medicine and it is known to contain bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids, vitamins, minerals.

Citrullus lanatus has been reportedly used widely in traditional herbal medicine. The **leaves** of *Citrullus lanatus is* analgesic, anti-inflammatory, mosquitocidal, gonorrhoea and anti microbial property. The **fruits** of *Citrullus lanatus* are eaten as a febrifuge when fully ripe or even when almost putrid. The **fruit** is used as a diuretic, anti-cancer, for treatment of high BP, antiviral and is effective in the treatment of dropsy and renal stones. The **seed** is also a good vermifuge and has a hypotensive action. It is demulcent, pectoral and tonic. It is sometimes used in the treatment of the urinary tract infections as well as bed wetting. The **root** is purgative and in high dose it can also serve as emetic. **Fatty oil in the seed**, as well as aqueous or alcoholic extracts, had been reported to paralyze tapeworms and roundworms. The **rind** of the fruit is prescribed in cases of alcoholic poisoning and diabetes. *Citrullus lanatus* is used in Northern Sudan for burns, swellings, rheumatism, gout and as laxative.

The biological activities reviewed include antimicrobial, antioxidant, anti-plasmodial, anti-inflammatory, anti-prostatic hyperplasia activity, antigiardial activity, anti-oxidant, analgesic properties, its effects on the histology of the kidney of adult Wistar rats, antisecretory, antidiabetic, laxative, antiulcerogenis and hepatoprotective activities. In view of its wide pharmacological and biological activities, it's traditionally reported therapeutic potential such as, antihypertensive, anti diarrhoeal, as well as its in-depth toxicity studies, among others, are yet to be experimented.

The species of *Citrullus* such as *Citrullus colocynthis* have already reported anti-cancer (breast cancer) activity.

Based on the ethnomedical information and studies available, the present research work has been framed to carry out the following studies on the leaves of *Citrullus lanatus.*

I. Pharmacognostical Evaluation

- ❖ Macroscopical evaluation and Microscopical Evaluation.
- ❖ Microscopical evaluation
- ❖ Standardization parameters
- ❖ Quantitative Analytical parameters.
- ❖ Powder Microscopy and Fluorescence analysis of powder and extracts.

II. Phytochemical Evaluation

- ❖ Preliminary phytochemical screening.
- ❖ Quantitative estimation of some secondary metabolites present in the plant.
- ❖ TLC and HPTLC finger print analysis.

III. Pharmacological evaluation

1. *In-vitro* antioxidant activity by various methods
   - ❖ **S**cavenging of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical
   - ❖ Total antioxidant activity by Phosphomolybdenum Method.
   - ❖ Reducing power assay
   - ❖ Ferric Reducing Antioxidant Power Assay (TPTZ method).
2. Larvicidal activity of methanolic extract of *Citrullus lanatus.*
3. *In vitro* activity of chicken pancreatic lipase inhibition Assay.
4. *In vitro* anti-cancer (Breast cancer) activity by MTT assay.

5. *In vitro* Anti-diabetic activity by various methods

- Non-enzymatic glycosylation of haemoglobin Assay.
- Glucose uptake in yeast cells
- % inhibition of Glucose uptake in 5mM and 10mM glucose concentrations
- Alpha amylase inhibition assay.
- Alpha glucosidase inhibition assay

PLANT PROFILE

CHAPTER IV

PLANT PROFILE[56-59]

DESCRIPTION

It is an annual climbing or trailing herb, with hairy stem up to 10m long. Tendrils divided at the tip into two or three parts. Separate male and female flowers are borne on the same plant.

Botanical Source: *Citrullus lanatus* (Thunb). Matsum. & Nakai

Family: Cucurbitaceae

Synonyms: *Citrullus vulgaris* Schrad., *Colocynthis citrullus* Linn., *Citrullus citrullus* (L.), *Cucubertia citrullus* L., *Anguria citrullus* Mill., *Momordica lanata* Thunb.

Common Names: Watermelon, wild watermelon, sweet melon (English); Egusi melon (English, Kenya); pastèque, melon d'eau (French).

Vernacular Names

Malaysia : Tembikai

English : Watermelon

India : Karingda

Chinese : Da zi gua zi xi gua.

Tamil : Pitcha.

Sanskrit : Tarambuja.

Hindi : Tarbuj

General

Symbol : CILAL

Group : Dicot

Family : Cucurbitaceae

Duration : Annual

Growth habit : Vine Forb/ herb

GEOGRAPHY & DISTRIBUTION: *Citrullus lanatus* is thought to be native to Africa. It is found in grassland and bushland, mostly on sandy soils, and often along watercourses or near water, up to 1,785 m above sea level. It flourishes in dry climates and requires only limited rainfall. Some say that the Kalahari region (Botswana, Namibia and South Africa) as the area of origin, whereas others suggest it is native to north eastern Africa.

HABITAT: Grassland and bushland, often along watercourses.

Classification:

Kingdom : Plantae - Plants

Subkingdom : Tracheobionta - Vascular plants

Superdivision : Spermatophyta - Seed plants

Division : Magnoliophyta - Flowering plants

Class : Magnoliopsida – Dicotyledons

Subclass : Dilleniidae

Order : Violales

Family : Cucurbitaceae

Genus : Citrullus Schard - watermelon

Species : *Citrullus lanatus (Thunb.) Mastum. & Nakai var lanatus*

ETHNOMEDICAL USES

(1) *Citrullus lanatus* has been reportedly used widely in traditional herbal medicine. The fruits of *Citrullus lanatus* are eaten as a febrifuge when fully ripe or even when almost putrid. The fruit is also diuretic and is effective in the treatment of dropsy and renal stones.

(2) The root is purgative and in high dose it can also serve as emetic.

(3) The seed is demulcent, pectoral and tonic. It is sometimes used in the treatment of the urinary tract infections as well as bed wetting. The seed is also a good vermifuge and has a hypotensive action.

(4) Preliminary research indicates that the consumption of watermelon may have antihypertensive effects

(5) Fatty oil in the seed, as well as aqueous or alcoholic extracts have been reported to paralyze tapeworms and roundworms.

(6) The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes

(7) *Citrullus lanatus* is used in Northern Sudan for burns, swellings, rheumatism, gout and as laxative.

(8) The fruits are used as a drastic purgative in Senegal; they are also used to treat diarrhoea and gonorrhoea in Nigeria.

(9) Tar is extracted from the seeds and used for the treatment of scabies and for skin tanning. The seed oil has an anthelmintic action which is better than that of pumpkin seed oil.

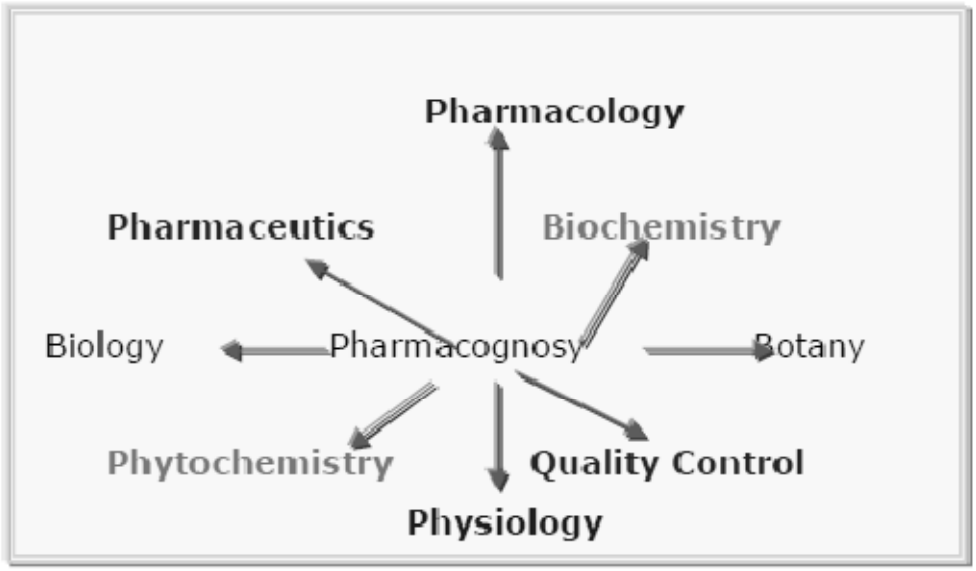
PHARMACOGNOSTICAL EVALUATION

CHAPTER V

PHARMACOGNOSTICAL STUDIES

Pharmacognosy is the study of medicines derived from natural sources. The study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources is the definition given by The American Society of Pharmacognosy. It is the oldest of all pharmacy sciences. The name "Pharmacognosy was "derived from the Greek Pharmacon, a drug, and gignosco, acquire knowledge (the entire meaning of drugs). Pharmacognosy is related to both botany and plant chemistry "Phytochemistry ", and its history entitles it to be regarded as parent of both. [60,61]

Fig: 1. Pharmacognosy is Parent for all



At present pharmacognosy involves the study of crude drugs and their natural derivatives like *Digitalis* and its glycoside, digoxin; *Datura* and its alkaloid, atropine; Opium and its purified compound morphine.

Pharmacognostical evaluation represents valuable information regarding the morphology, microscopical and physical characters of crude drugs and thus gives the scientific information regarding the purity and quality of crude drugs.

MATERIALS AND METHODS

SECTION A - MACROSCOPICAL STUDIES [61- 66]

Macroscopical studies include aspects of the outward appearance (shape, structure, colour and pattern) as well as the form and structure of the internal parts like cells etc. Some of these gross morphological characters of drugs such as shape, size, margin, apex and venation are identification features of drugs. these features give valuable information about the drugs.

Collection of plant material

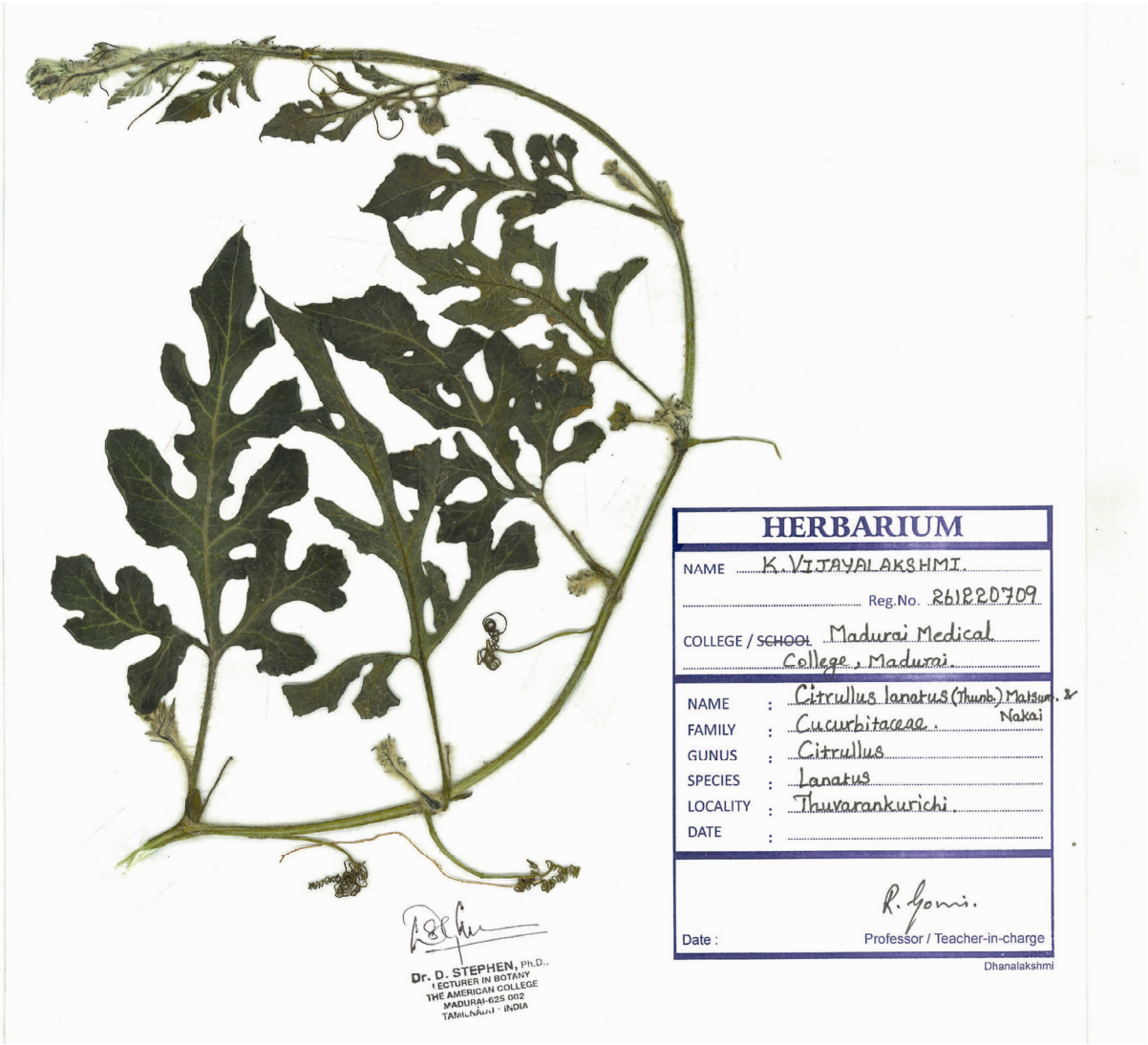
The leaves of *Citrullus lanatus* have been collected in Thuvarankurichi during the month of August 2013. The plant was collected and authenticated by Dr. Stephen, Senior Lecturer in Botany and Taxonomist, American College, Madurai. The authenticated herbarium of plant has been kept in the Department of Pharmacognosy, Madurai Medical College, Madurai. A copy of herbarium is shown in **Fig 2**.

The leaves of *Citrullus lanatus* were collected and the macroscopical characters like shape, structure, colour and pattern were studied. The photographic representations of the macroscopic features are presented in **Figs 3.**

SECTION B - MICROSCOPICAL STUDIES [67-76]

Microscopical evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, and in detection of

Fig.2: Herbarium of *Citrullus lanatus (Thunb.) Matsum. & Nakai.*



adulterants (eg. insects, animal faeces, mould, fungi etc.) as well as identifying the plant by characteristic tissue features. Every plant possess a characteristic tissue structure, which can be demonstrated through study of tissue arrangement, cell walls, and configuration when properly mounted in stains, reagents and media.

The microscopical evaluation allows more detailed examination of the plant material to identify the organised drug by its histological character. It provides detailed information about the crude drugs by virtue of its property to magnify the fine structures of minute objects to be visualised and thereby confirm the structural details of the plant drugs under evaluation. It can also be used in the determination of the optical as well as micro chemical properties of the crude drug confirmation study.

Collection of specimens

The plant specimen for the proposed study was collected from *Citrullus lanatus* leaf. Care was taken to select healthy plant. The leaf was cut and removed from the plant and fixed in FAA (Formalin- 5ml + Acetic acid- 5ml + 70% Ethyl alcohol-90 ml) After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass, 1940.[67] Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60˚C) until TBA solution stained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary **Microtome.** The thickness of the sections was 10-12µm. Dewaxing of the sections was by customary procedure (Johansen, 1940)[68]. The sections were stained with **Toluidine blue** as per the method published by O'Brien et al. (1964)[69]. Since **Toluidine blue** is a polychromatic stain. The staining results were remarkably good; and some **cytochemical**

reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the **lignified** cells, dark green to suberin, violet to the mucilage, blue to the **protein** bodies etc. whenever necessary sections were also stained with **safranin** and **Fast green** and iodine in potassium iodide.

For studying the stomatal morphology, venation pattern and trichome distribution, **paradermal sections** (sections taken parallel to the surface of leaf) as well as **clearing** of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jaffrey's maceration fluid (Sass, 1940) were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with **Nikon labphoto 2** microscopic Unit. For normal observations **bright field** was used for the study of **crystals, starch grains,** and **lignified** cells, polarized light was employed. Since these structures have **birefringent property,** under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Easu, 1964)[77]. The microscopic features observed for both plant are represented in **Fig. 4** to **7.**

SECTION C - QUANTITATIVE MICROSCOPY[76-78]

Quantitative analytical microscopy is useful in measuring the cell contents of the crude drugs and help in their identification, characterization and standardization. A clear idea

about the identity and characteristic features of the drug can be obtained after several numbers of determinations. The number so obtained can be compared with a standard value to find out whether it is within the range. It helps to determinine the purity of the plant material.

LEAF CONSTANTS

The stomatal number, stomatal index, vein islet number and vein termination number were determined on fresh leaves using the standard procedures. A number of leaf measurements are used to distinguish between some closely related species not easily characterised by general microscopy.

Determination of stomatal number and stomatal index

Stomatal number: Stomatal number is the average number of stomata/sq.mm of epidermis of the leaf.

Stomatal index: Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells.

To study the morphology (type of stomata),stomatal number and stomatal index of leaf, the leaf was subjected to epidermal peeling.

Procedure: The leaf was cleared by boiling with chloral hydrate solution. The upper and lower epidermis was peeled out separately by means of forceps. The cleared leaf was placed on the slide and mounted in glycerin. A camera Lucida and drawing board was placed and stage micrometer was inserted for making the drawing scale. A square of 1mm was drawn by means of stage micrometer. The slide with cleared leaf (epidermis) was placed on the stage of the microscope and examined under 45X objective and 10X eye piece. The epidermal cell and stomata was traced .The number of stomata present in the area of 1sq.mm

including the cell or at least half of its area within the square was counted. The average number of stomata per sq.mm was determined and their values are tabulated.

For stomatal index, the glycerin mounted leaf peeling as mentioned above was made and circle like mark for each stomata and cross like mark for each epidermal cells was marked on the chart paper. The stomatal index was calculated by using the formula - Stomatal **index I= S/ (E+S) X 100;** where I is the stomatal index, S is the number of stomata in 1sq.mm area of leaf and E is the number of epidermal cells in 1sq.mm area of leaf. The values are tabulated.

Determination of vein islet and vein termination number

Vein islet number is defined as the number of vein islet per sq.mm of the leaf surface midway between the midrib and the margin. It is used to denote the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands.

Vein termination number is defined as the number of vein termination per sq.mm of the leaf surface midway between the midrib and margin. A vein termination is the ultimate free termination of a veinlet or branch of a veinlet.

Procedure: A few leaves were boiled in chloral hydrate solution in a test tube placed in a boiling water bath until clear. The cleared leaves were stained with saffranin solution and a temporary mount was prepared with glycerin solution. The stage micrometer placed on the microscopic stage, examined under 10X objective and 6X eye piece and an area of 1sq.mm square was drawn. The cleared leaf piece was placed on the microscope stage, the vein islets and vein terminals included in the square was drawn. The number of vein islets and vein terminals within the square were counted. The results obtained for the number of vein islets and vein terminals in 1sq.mm are tabulated in **Table 1**.

SECTION D - STANDARDIZATION PARAMETERS[77-80]

The determination of the foreign organic matter, loss on drying, ash values and extractive values etc. gives a clear idea about the specific characteristics of crude drug under examination, besides its macro-morphological or cyto-morphological, microscopical nature in both its entire and its powder form. These diagnostic features enable the analyst to know the nature and characteristic of crude drugs.

FOREIGN ORGANIC MATTER

The part of organ or organs other than those specified in the definition or description of the crude drugs is defined as foreign organic matter.

Procedure: 500g of the original sample was weighed and spread out in a thin layer and inspected with the unaided eye or with the use of a 6X lens and the foreign organic matter was separated manually as completely as possible. The foreign organic matter was weighed and the percentage of foreign organic matter was determined from the weight of the drug taken. The results obtained are presented in **table 2.**

ASH VALUES

The ash values for air dried powdered leaves of *Citrullus lanatus* were determined as per official method. The determination of ash is useful for detecting low grade products, exhausted drug and excess of sandy or earthy matter.

Different types of ash values are used in detection of crude drugs like, total ash, acid insoluble ash, water soluble ash and sulphated ash.

Determination of total ash

Total ash is useful in detecting the crude drugs that are mixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drug with different inorganic contents to improve their appearance.

Procedure: 2 to 3g of the air dried crude drug was weighed accurately and taken in a tared platinum or silica dish and incinerated at a temperature not exceeding 450^{o}C until free from carbon then cooled and weighed. The percentage of ash with reference to the air dried drug was calculated. The results obtained are presented in **table 2.**

Determination of acid insoluble ash

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure

The ash obtained from the total ash was boiled for 5min with 25mL of 2M hydrochloric acid; the insoluble matter was collected in a Gooch crucible or on an ashless filter paper and washed with hot water and ignited, cooled in a dessicator and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug. The results obtained are presented in **table 2.**

Determination of water soluble ash

Water soluble ash is used to detect the presence of material exhausted by water. If carbon is still present after heating at a moderate temperature, the water- soluble ash may be separated and the residue again ignited.

Procedure

The ash obtained from the total ash was boiled for 5min with 25mL of water; the insoluble matter was collected in a Gooch crucible or on an ashless filter paper and washed with hot water and ignited for 15min at a temperature not exceeding 450^{o}C. The weight of the insoluble matter was subtracted from the weight of the total ash; the difference in weight representing the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug. The results obtained are presented in **table 2.**

Determination of sulphated ash

The treatment of the drug with sulphuric acid before ignition, whereby all oxides and carbonates are converted to sulphates is called as sulphated ash.

Procedure

A platinum dish was heated to redness for 10min and allowed to cool in a desiccator and weighed. 1g of the substance being examined was placed in the dish, moistened with sulphuric acid, ignited gently, moistened again with sulphuric acid and ignited at about 800°C. It was then cooled and weighed. The percentage of sulphated ash was calculated with reference to the air dried drug. The results obtained are presented in **table 2.**

LOSS ON DRYING

The moisture content of a drug should be minimized to prevent decomposition of plant material due to chemical or microbial contamination. It may be determined by heating a material at constant temperature to constant weight.

Procedure

About 2g of the powdered crude drug was accurately weighted in a tared dish and dried in an oven at 100˚-105˚ C. It was cooled in a desiccator and again weighed. The loss on

drying was calculated with reference to the amount of the dried powder taken and the results obtained are presented in **table 2.**

EXTRACTIVE VALUES

The extractive values are the important factor to determine the amount of active principle or phytoconstituents present in the plant materials, when extracted with suitable solvents. The extraction of crude drug with a particular solvent yields a solution containing different phyto-constituents. The composition of these phyto constituents in that particular solvent depends upon the nature of the drug and solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of a particular drug sample; for example, in a drug where the extraction procedure for the constituents commences with water as the solvent, any subsequent aqueous extraction on the re-dried residue will give a very low yield of soluble matter.

Determination of water soluble extractive

Procedure: 5g of the air dried drug, coarsely powdered have to be macerated with 100mL of water closed flask for 24h, shaking frequently during the first 6h and allowing to stand for 18h. Thereafter, filter rapidly taking precautions against loss of water, evaporate 25 mL of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 105°C and weigh. The percentage of water soluble extractive with reference to the air dried drug was calculated.

Determination of ethanol soluble extractive

Procedure

Macerate 5g of the air dried drug, coarsely powdered, with 100mL of the ethanol of the specified strength in a closed flask for 24h, shaking frequently during the first 6h and allowing to stand for 18h. Thereafter, filter rapidly taking precaution against ethanol, evaporate 25mL of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 105°C

and weigh. Calculate the percentage of ethanol soluble extractive with reference to the air dried drug.

Determination of ethanol soluble extractive

Procedure

Macerate 5g of the air dried drug, coarsely powdered, with 100mL of the methanol of the specified strength in a closed flask for 24h, shaking frequently during the first 6 hours and allowing to stand for 18h. Thereafter, filter rapidly taking precaution against ethanol, evaporate 25mL of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 105°C and weigh. Calculate the percentage of methanol soluble extractive with reference to the air dried drug.

Determination of petroleum ether soluble extractive

The procedure adopted under ethanol soluble extractive was followed using petroleum ether as a solvent.

Determination of hexane soluble extractive

The procedure adopted under ethanol soluble extractive was followed using hexane as a solvent.

Determination of chloroform, benzene, ethyl acetate and acetone soluble extractive

The procedure adopted under ethanol soluble extractive was followed using the requisite solvent as the medium of extraction.The results obtained for the various extractive values are presented in **table 2.**

FOAMING INDEX

Some plant materials when shaken with water cause persistent foam which may be attributed to the presence of saponins in that material. The foaming ability of an aqueous solution of plant materials and their extracts is measured in terms of foaming index.

Procedure

An accurate quantity of about 1g of the coarse plant material was weighed and transferred into an Erlenmeyer flask containing 100mL of boiling water. The flask was boiled at moderate heat for 30min. The solution was cooled and filtered into a 100mL volumetric flask and sufficient distilled water was added to dilute to volume. The solution was poured into ten stoppered test tubes in successive portions of 1mL, 2mL, etc. upto 10mL, and the volume of the liquid in each tube was adjusted with water upto 10mL. The tubes were then stoppered and shaken in a length wise motion for 15sec (two shakes/sec) and allowed to stand for 15min. The height of foam was measured. If the height of the foam in every tube was less than 1cm the foaming index was less than 100. If a height of foam of 1cm was measured in any test tube, the volume of the plant material decoction in this tube (a) was used to determine the index. If the height of the foam was more than 1cm in every tube, the foaming index was over 1000. In this case, the determination was repeated using a new series dilution of the decoction in order to obtain a result. The foaming index was calculated by using the following formula 1000/A where A was the volume in mL of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm was observed. The results obtained are presented in **table 2.**

SWELLING INDEX

Swelling index is the volume in mL taken up by the swelling of plant material under specified conditions. The medicinal plant materials like gums, mucilage, and pectin have swelling property.

Procedure: An accurately weighed 1g of the powdered drug material was taken in the 25mL glass stoppered measuring cylinder. 25mL of water was added and shaken the mixture thoroughly every 10min for 1h. Then, allowed to stand for 3h at room temperature. The volume in mL occupied by the plant material was measured, including sticky mucilage was observed. The results obtained are presented in **table 2.**

SECTION E - POWDER ANALYSIS & MICROSCOPY[81-83]

The powdered crude drug analysis was aimed to study and also to assess the quality of herbal drugs for therapeutic value which are generally studied by classical pharmacognostical studies. The authenticity of herbal drugs was confirmed by comparison of their powder characteristics.

Procedure

A) Reaction of chemicals with powdered crude drugs

The raw leaf powder of C*itrullus lanatus* was treated with different chemical reagent such as iodine solution, 10% potassium hydroxide solution, glacial acetic acid etc. on a clean watch glass for the identification of secondary metabolites. The colours obtained with various reagents are presented in **Table 3.**

B) Fluorescence analysis

The fluorescence nature of powder drugs was analysed to find out whether any fluorescent compound was present in the sample and the observations with different chemicals were also carried out and recorded. The air dried plant materials of both plants

were taken in clean warch glass and subjected to different chemicals such as acids, alkalis and some reagents are observed under day light and UV light. Detailed fluorescence behavior of crude drug powder has been shown in **Table 4.**

C) Powder microscopy

The dried leaf was powdered and the powder was passed through sieve no.60 for the study of powder microscopy. Chloral hydrate, water, iodine, phloroglucinol and hydrochloric acid (1:1) etc. were employed as mounting medium. The pictorial representation are presented in **Fig 8.**

RESULTS AND DISCUSSION

SECTION A - MACROSCOPICAL EVALUATION

Macroscopical characters of *Citrullus lanatus (Thunb.) Matsum. & Nakai*

Whole plant: *Citrullus lanatus* is an annual climbing or trailing herb, with hairy stem up to 10 m long. Tendrils divided at the tip into two or three parts. Separate male and female flowers are borne on the same plant. (**Fig. 3.1)**

Leaves: Leaf blades up to about 20 × 20 cm, more or less hairy, usually deeply 3–5-lobed, the central lobe being the largest. The lobes themselves are further divided. Leaf stalks (petioles) up to about 19 cm long, more or less hairy. **(Fig. 3.2, 6, 7)**

Flowers: Solitary, borne in leaf axils. Both male and female flowers are yellow, up to 3 cm in diameter, and borne on pedicels (flower stalks) up to 45 mm long. Flowers are usually pollinated by honey bees. **(Fig. 3.3)**

Fruits: Fruits of wild plants up to about 20 cm in diameter, greenish mottled with darker green. Fruits of cultivated plants up to about 70 × 30 cm, rounded, oval or oblong, with a golden-yellow to dark green skin, the skin being uniform, mottled or striped. Flesh usually red or yellow, sometimes orange, pink or white. (**Fig.3.4)**

Seeds: Flat, smooth, variable in size and colour (white, tan, brown, black, red, green or mottled).(**Fig. 3.5)**

SECTION B - MICROSCOPICAL EVALUATION

Anatomy of the leaf: The leaf has very thick abaxially hanging midrib and thin lamina **(Fig.4.1).** The midrib is 1.9mm thick and 1.7mm wide. It has four thick ridges alternatively deep furrows. **(Fig.4.1)** The adaxial part of the midrib has short, tick cone. The epidermis has

Fig. 3.1. HABITAT AND WHOLE PLANT OF

Citrullus lanatus (Thunb.) Matsum. & Nakai

Fig. 3.2. LEAF OF *Citrullus lanatus (Thunb.) Matsum. & Nakai*

Fig. 3.3. FLOWER OF *Citrullus lanatus (Thunb.) Matsum. & Nakai*

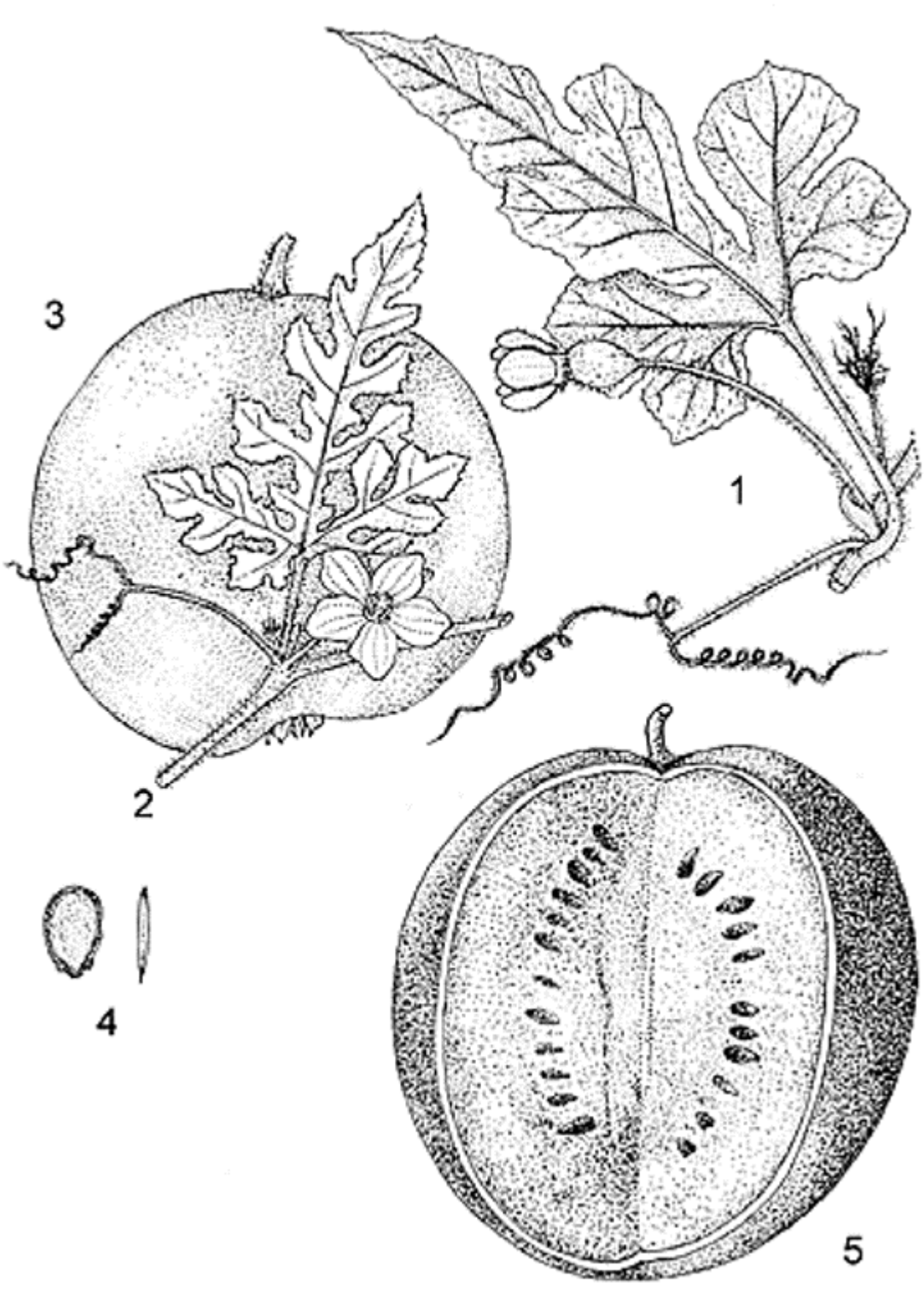
Fig. 3.4. FRUITS OF *Citrullus lanatus (Thunb.) Matsum. & Nakai*

Fig. 3.5. SEEDS OF *Citrullus lanatus (Thunb.) Matsum. & Nakai*

Fig. 3.6. DORSAL VIEW OF *Citrullus lanatus (Thunb.) Matsum. & Nakai* LEAF.

Fig. 3.7. VENTRAL VIEW OF *Citrullus lanatus (Thunb.) Matsum. & Nakai* LEAF.

Fig. 3.8. LINE DRAWING OF *Citrullus lanatus (Thunb.) Matsum. & Nakai.*



thin intact epidermal layer of rectangular cells. Inner to the epidermis is a narrow, two or three layers of collenchyma and the remaining ground tissue is thin walled compact parenchyma cells.

The vascular system of the midrib is multi stranded. There is a large abaxial median bundle, two adaxial lateral smaller bundles and cone still smaller adaxial median bundles **(Fig. 4.1)** All the bundles are **bicollateral** having phloem strands both on the outer and inner sides of the xylem. The xylem consists of short radial chains of wide circular elements. Phloem strands are composed of small groups of sieve elements which are small and darkly stained. **(Fig.4.2, 3).**

The lateral vein is simple in structure. It projects slightly on the lower side and the upper side is flat. **(Fig. 5.1)** The epidermal cells of the lateral veins are large and thick; they are circular or cylindrical and thin walled. The vascular bundle is small, collateral and includes adaxial group of angular xylem elements and small cluster of abaxial phloem elements.

Lamina (Fig. 5.2): The lamina is 90-100µm thick. It is heterofacial with distinct adaxial sides. It is heterofacial with distinct adaxial and abaxial side. It is amphistomatic with stomata located on the adaxial and abaxial side.The epidermal cells are small elliptical or rectangular and thin walled. The palisade zone consists of single layer of cylindrical cells which are loosely arranged. The sponge parenchyma cells small and spherical. They form wide air chambers separated by partition filaments. **(Fig. 5.2)**

Stomata (Fig.6.1, 2, 3): The stomata epidermal cells were studied in paradermal sections of the lamina. The epidermal cells small and vary in shape and size. Their anticlinal walls are thick and wavy. The stomata are diffuse in distribution. **(Fig.6.1,2)** The stomatal type is **actinocytic. (Fig.6.2, 3)** The stoma is surrounded by three to six subsidiary cells which

Fig.4.1: T.S. of leaf through midrib

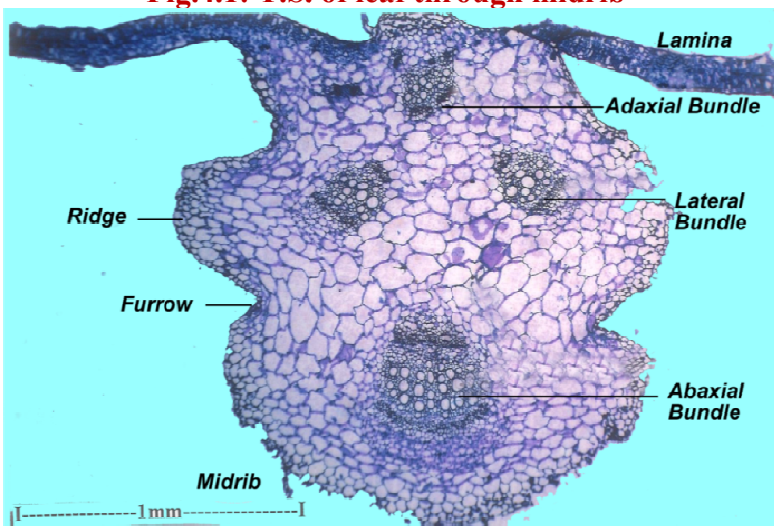


Fig. 4.2: Adaxial part of the midrib – enlarged

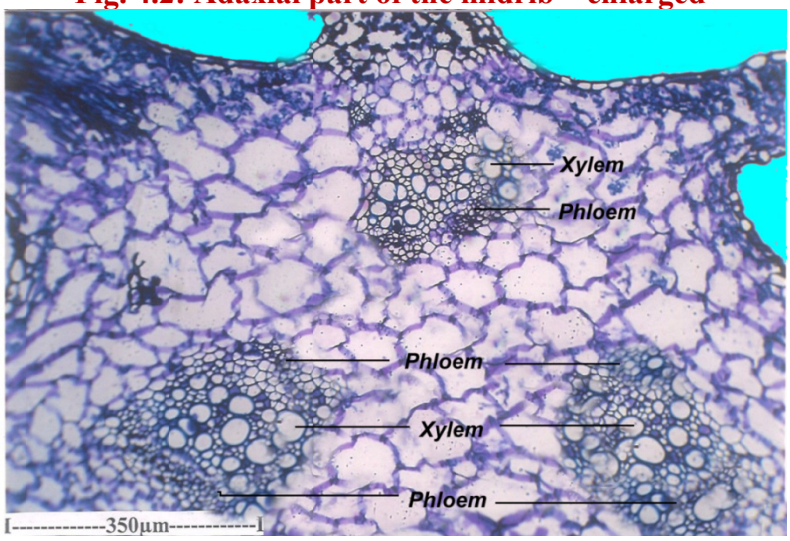


Fig.4.3: Abaxial part of the midrib – enlarged

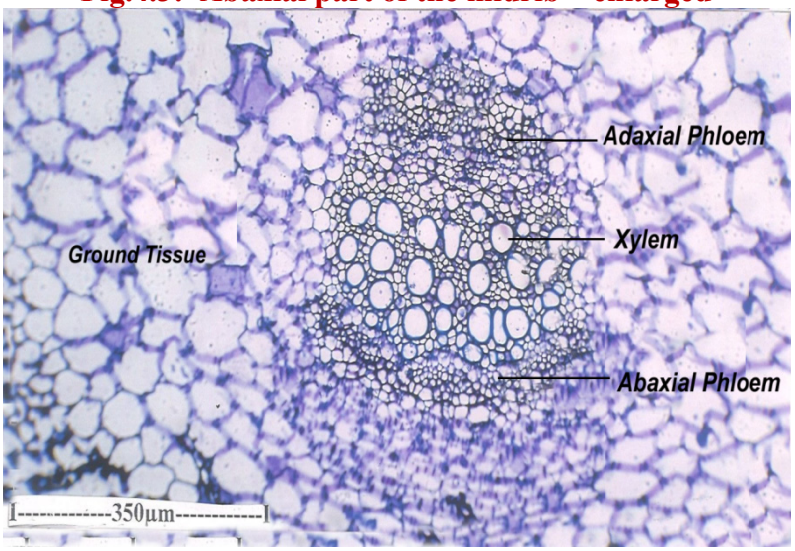


Fig. 5.1 : T.S. of Lamina through lateral vein

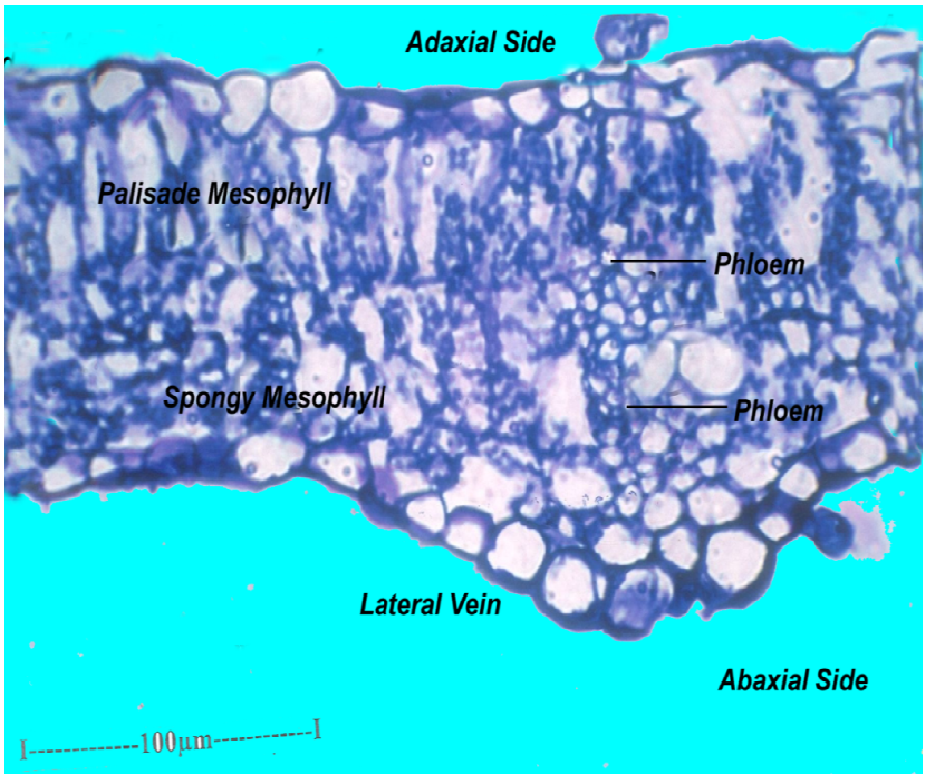


Fig. 5.2: T.S. of Lamina

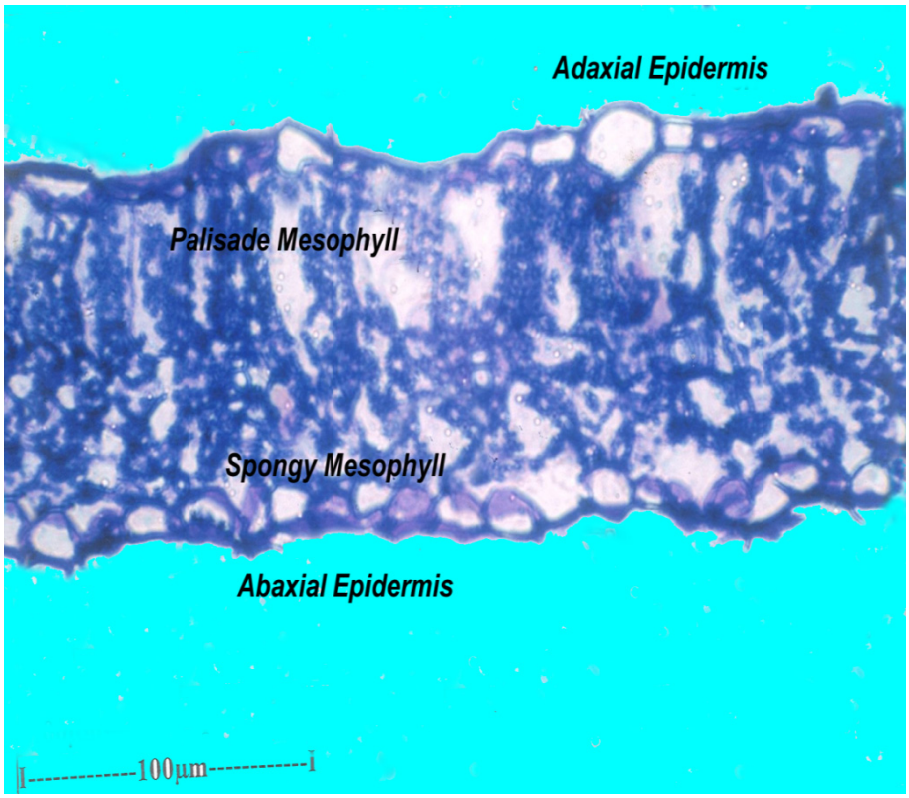


Fig. 6.1: Paradermal section of the lamina

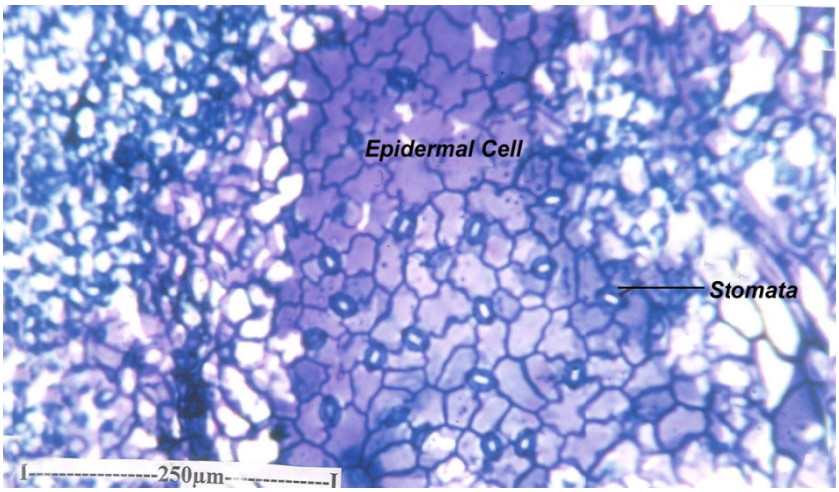


Fig.6.2: Stomata enlarged

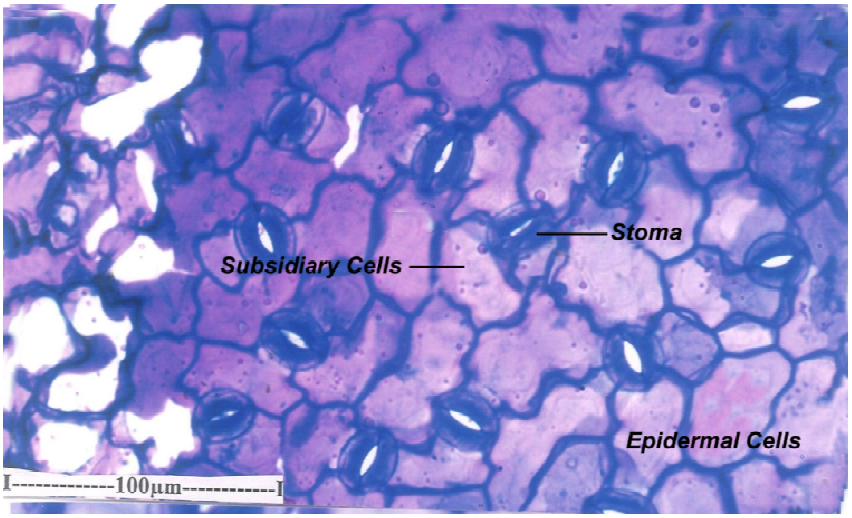


Fig. 6.3: One stomata with radiating subsidiary cells

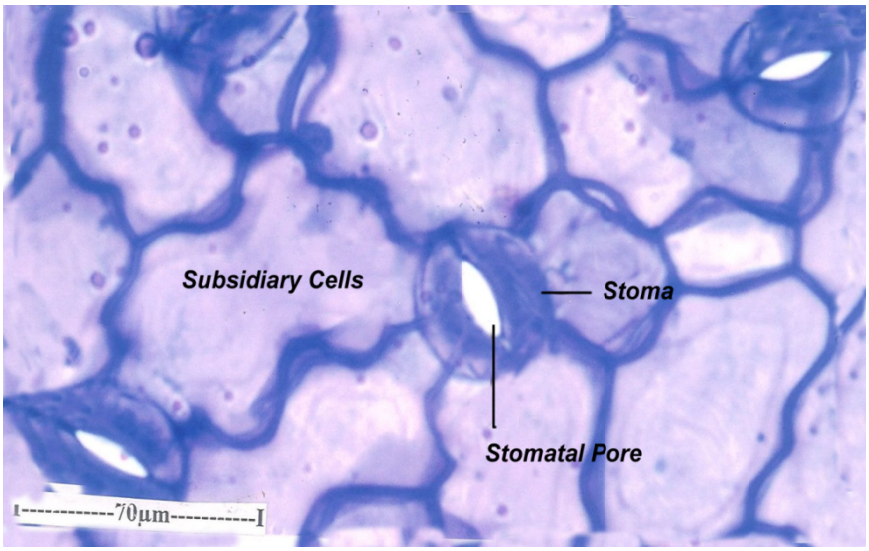


Fig. 7.1: Reticulate venation pattern of the lamina

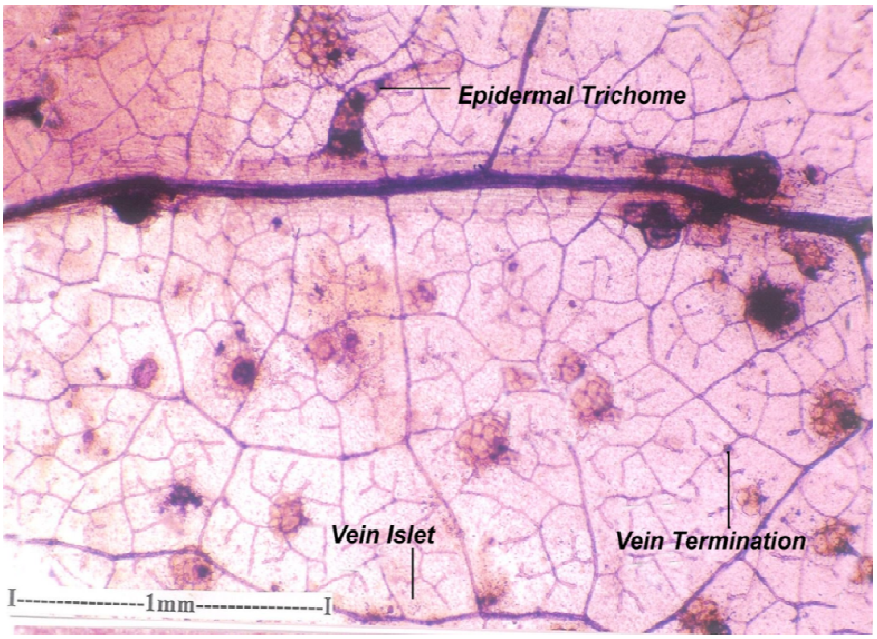
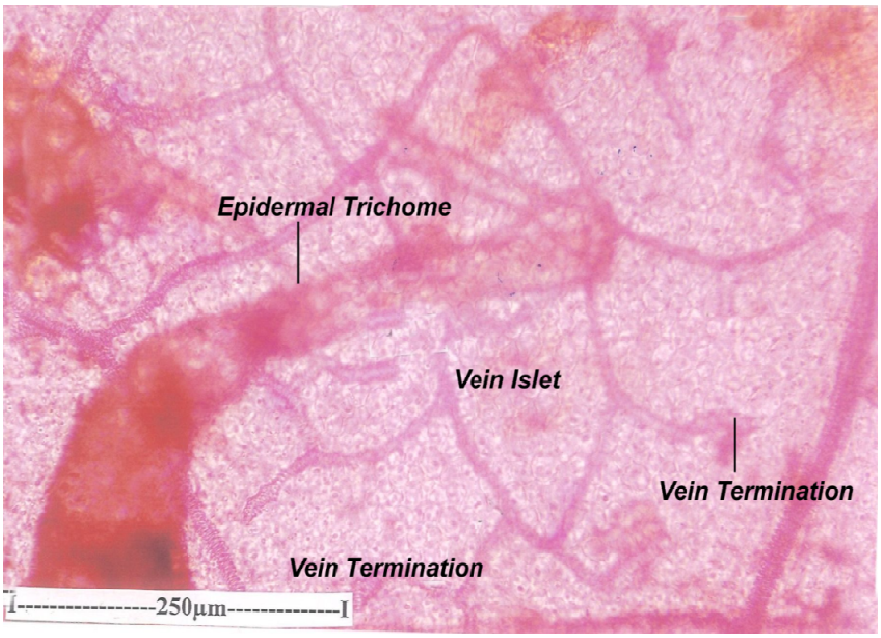


Fig. 7.2: Venation showing vein islet and vein termination



radiate from the guard cells. **(Fig.6.2,3)** The guard cells are broadly elliptical and are 15x20µm in size.

Venation pattern (Fig.7.1,2): The venation is **reticulate type.** The reticulation is dense. The veins are thin and straight. The **vein-islets** are distinct and are surrounded by thin and straight veins. The **vein terminations** are either triple (unbranched) or branched forming tree like outline. **(Fig7.2.).** Multi cellular, uniseriate unbranched epidermal trichomes are occasionally seen on the epidermis. The trichome has spherical terminal cells. The trichome is 450µm long.

SECTION C -QUANTITATIVE ANALYTICAL MICROSCOPY

The results obtained for the determination of leaf constants are presented in **Table.1.**

Table.1 : Quantitative analytical microscopical parameters of the leaf of Citrullus *lanatus*

S. No.	Parameters*	Values obtained
1	Stomatal number in upper epidermis	21.83 ± 0.300
2	Stomatal number in lower epidermis	32.25 ± 0.629
3	Stomatal index in upper epidermis	19.68 ± 0.396
4	Stomatal index in lower epidermis	22.12 ± 0.093
5	Vein islet number	12.66 ± 0.333
6	Vein termination number	19.66 ± 0.881

***mean of three readings ± SEM**

From the table, it can be observed that the number of stomata in the upper epidermis was found to be 21.83 ± 0.300 while in the lower epidermis it was 32.25 ± 0.629. The stomatal index in upper epidermis and lower epidermis was 19.68 ± 0.396 and 22.12 ± 0.093 respectively. The vein islet number was found to be 12.66 ± 0.333 and the vein termination number was 19.66 ± 0.881. The values help in identification of leaf of *Citrullus lanatus* from the species of the genus *Citrullus* since these values are unique for each plant.

SECTION D - STANDARDIZATION PARAMETERS

The results obtained for various standardization parameters are presented in **Table 2**. From the **table 2**, it can be seen that the foreign organic matter present in the crude material was very low. The percentage of total ash was found to be 14.73 ± 0.080 and the percentage of water soluble ash was found to be 4.03 ± 0.062 while the acid insoluble ash was 1.28 ± 0.074 and the percentage of sulphated ash was found to be 17.76 ± 0.292. The determination of ash values helps to find out where the powdered material was adulterated with sand and other inorganic material. The water soluble ash helps us to find the amount of inorganic material present in the crude drug, while acid insoluble ash helps us to find the amount of sand and other debris in the crude material.

The various extractive values with different solvents have been determined. A maximum extractive value was found with methanol (26.57 ± 0.268) followed by water (24.99 ± 0.671). The extractive values helps us to decide what solvent will be useful for extraction of maximum active principle and also helps to decide whether the crude material has already been exhausted or not.

Table.2 : Standardization parameters of *Citrullus lanatus*

S. No	Parameters*	Values* expressed as %
1	**Foreign organic matter**	0.04 ± 0.180
2	**Loss on drying**	7.29 ± 0.012
3	**Ash value**	
	Total ash	14.73 ± 0.080
	Acid insoluble ash	1.28 ± 0.074
	Water soluble ash	4.03 ± 0.062
	Sulphated ash	17.76 ± 0.292
4	**Extractive values**	
	Petroleum ether	29.33 ± 0.360
	Chloroform	7.39 ± 0.101
	Ethyl acetate	8.09 ± 0.210
	Ethanol	13.65 ± 0.413
	Methanol	26.57 ± 0.268
	Water	24.99 ± 0.671
	Acetone	11.25 ± 0.145
	Benzene	5.87 ± 0.153
	Hexane	5.64 ± 0.046
5	Foaming index	<100
6	Swelling index	2.4 ± 0.493(ml)

***mean of three readings ± SEM**

The pharmacognostic evaluation which includes macroscopical, microscopical, analytical parameter evaluation helps in identity, quality and purity of the plant material either as a whole or in the form of powder.

SECTION E - POWDER ANALYSIS & MICROSCOPY

The powdered crude drug analysis was aimed to study and also to assess the quality of herbal drugs for therapeutic value which are generally studied by classical pharmacognostical studies. The authenticity of herbal drug was confirmed by comparison of their powder characterics. The reaction of various chemical reagents is tabulated in **Table 3.**

Table 3: Reaction of chemical with powdered drug *Ctrullus lanatus* with various chemical reagents

Drug powder + reagent	Colour in day light (Visible)	Colour in UV light	
		254nm	365nm
Powder	Yellowish green	Dark green	Yellowish green
Powder + 1M sodium hydroxide	Yellowish green	Fluorescent green	Dark green
Powder + Iodine	Yellowish green	Dark green	Dark brown
Powder + 10% potassium hydroxide	Yellow	Fluorescent green	Dark brown
Powder + 1M hydrochloric acid	Yellowish brown	Dark green	Dark brown
Powder + Glacial acetic acid	Yellowish green	Green	Orange
Powder + 50% sulphuric acid	Yellowish green	Dark green	Dark brown
Powder + 50% nitric acid	Brown	Greenish	Dark brown
Powder + 50% Hydrochloric acid	Yellowish brown	Dark green	Dark brown

Note : Colour reactions are viewed under natural light by naked eye

From the table, it can be observed that the powder with 10% potassium hydroxide and 1M sodium hydroxide under UV light at 254nm exhibited a fluorescent green which will be useful for identification of the plant material in crude form.

Fluorescent analysis of the Extracts

The behavior of various extracts in natural light and under UV light at 254nm and 365nmand presented in **Table.4**

From the table, it was observed that the ethanolic, methanolic, chloroform, benzene, petroleum ether, ethyl acetate, water, acetone and hexane extracts were orange colour under UV light at 365nm. The petroleum ether and water extracts were fluorescent green colour under UV light at 365nm. These parameters are useful for quality control and purity checking of the plant in powder form.

Table 4 : Fluorescence analysis of extracts of *Citrullus lanatus*

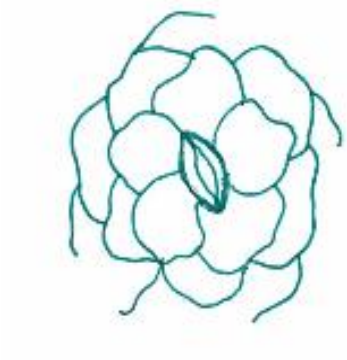
Extract	Consistency	Colour in day light (Visible)	Colour in UV light	
			254nm	365nm
Ethanol	Semisolid	Light green	Dark green	Orange
Methanol	Semisolid	Yellowish green	Dark green	Light orange
Benzene	Semisolid	Yellowish green	Dark green	Dark orange
Petroleum ether	Semisolid	Light yellow	Dark green	Fluorescent green
Ethyl acetate	Semisolid	Light green	Dark green	Dark orange
Water	Semisolid	Yellowish green	Greenish black	Fluorescent green
Chloroform	Semisolid	Light green	Green	Dark orange
Acetone	Semisolid	Light green	Green	Dark orange
Hexane	Semisolid	Light green	Dark green	Orange

Powder microscopy

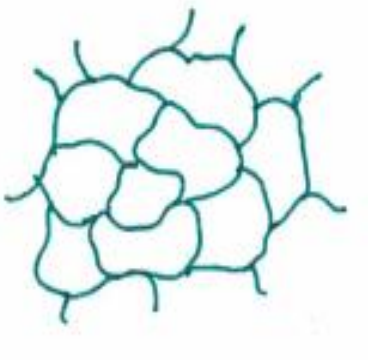
The various characteristic features observed when seen under a microscope are presented in **Fig. 8.** The features observed under a microscope include stomata, unbranched trichomes, xylem and phloem vessels.

Fig.8.: Powder Microscopy of Citrullus lanatus (Thunb.) Matsum. & Nakai

Actinocytic Stomata

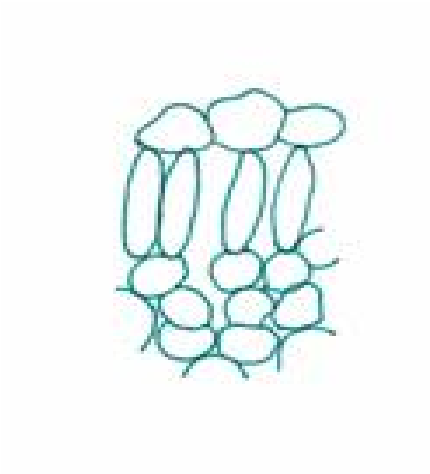


Fragments of Parenchyma Cells

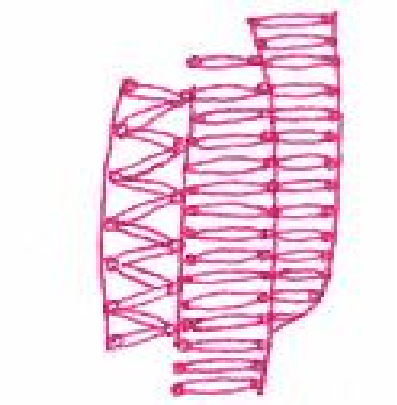


Multicellular uniseriate unbranched Epidermal Trichomes

Fragments of Palisade Cells



Xylem Vessels (Spiral and Annular)



PHYTOCHEMICAL EVALUATION

CHAPTER VI

PHYTOCHEMICAL EVALUATION

Phytochemicals means plant chemicals. They are naturally occuring in plants. They give plants its colour, flavour, smell and are part of a plant's natural defence system (disease resistance). The phytochemicals are bioactive, non-nutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major degenerative diseases. [84]

In plants phytochemicals attract beneficial and repel harmful organisms, serve as photoprotectants, and respond to environmental changes. They work together with nutrients and dietary fibres to protect the body against diseases, slow the aging process and reduce the risk of many diseases such as cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis and urinary tract infection. Alkaloids have analgesic effects, glycosides are used in cardiac diseases, tannins prevent urinary tract infection by preventing bacteria from adhering to the walls. Tannins, along with vitamin C help build and strengthen collagen. Saponins serve as natural antibiotics, which help body to fight infections and microbial invasions. They also enhance the effectiveness of certain vaccines, lower cholesterol and knock out some tumor cells. Flavonoids have antioxidant activity in biological systems and protect the body against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors The flavonoid quercetin is known for its ability to relieve hey fever, eszema, sinusitis and asthma while certain flavonoids also can protect low-density-lipoproteins from being oxidized, thereby playing an important role in atherosclerosis.[85]

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF EXTRACT

The leaves of *Citrullus lanatus* were collected in Thuvarankurichi during the month of August 2013. The leaves were washed thoroughly and dried in shade. The shade dried leaves were powdered and used for further studies.

Extraction of leaves of *Citrullus lanatu*s was carried out by washing the plants and drying at room temperature in 14 days. After that, they were filtered with sieve analyzer to get homogeneous particles and defatted with 2.5L of petroleum ether (60-80˚C) by cold maceration method for 72h. The solvent was then removed by filtration and the marc was dried. The dried marc was re-soaked with 2.5L of methanol. The steps were performed three times and the combined filtrates were evaporated to a cohesive mass using rota vapour.

SECTION A - PRELIMINARY PHYTOCHEMICAL SCREENING [86-90]

The preliminary phytochemical screening helps us in identifying the type of secondary metabolites present in plants. The screening was carried out Aqueous and methanolic extract of *citrullus lanatus* leaf powder. The various chemical tests carried out are described.

1. **Test for carbohydrates**

a. Molisch's Test: The aqueous extract of the powdered leaf when treated with alcoholic solution of α-naphthol in the presence of sulphuric acid. Purple colour indicates the presence of carbohydrates.

b. Benedict's test: The aqueous extract of the powdered was treated with Benedict's reagent and boiled on water bath and cooled. An orange colour precipitate indicates the presence of carbohydrates.

c. Fehling's Test: The aqueous extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. A red precipitate indicates the presence of free reducing sugars.

2. Test for Alkaloids

About 2g of the powdered material was mixed with 1g of calcium hydroxide and 5mL of water into a smooth paste and set aside for 5min. It was then evaporated to dryness in a porcelain dish on a water bath. To the residue, 20mL of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To the residue, 5mL of dilute hydrochloric acid was added. The solution was divided into four parts and 2mL of each of the following reagents were added and the colour noted below indicates the presence of alkaloids.

a) Mayer's Reagent - Cream precipitate

b) Dragendorff's Reagent - Reddish brown precipitate

c) Hager's Reagent - Yellow precipitate

d) Wagner's Reagent - Reddish brown precipitate

3. Test for phytosterols

The powdered leaf was first extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for phytosterols.

a. Salkowski's Test: A few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turning red indicates the presence of phytosterols.

b. Liebermann – Burchard's Test: To the chloroform solution a few drops of acetic anhydride and 1mL of concentrated sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring and the upper layer turning green indicates the presence of phytosterols.

4. Test for glycosides

The substance was mixed with a little anthrone on a watch glass. One drop of concentrated sulphuric acid was added, made into a paste and warmed gently over a water bath. A dark green coloration indicates the presence of glycosides.

Test for Cardiac Glycosides

a. Keller Killiani Test: The substance was boiled with 10% alcohol for 2min, cooled and filtered. To the filtrate, lead sub acetate was added and filtered. The filtrate was then extracted with chloroform. The chloroform layer was separated and evaporated to dryness. The residue was dissolved in glacial acetic acid with traces of ferric chloride. To this few drops of sulphuric acid was added slowly along the sides of the test tube. A reddish brown layer changes to green colour on standing.

b. Legal test: The substance was dissolved in pyridine, sodium nitroprusside solution was added to it and made alkaline. A pink or red colour indicates the presence of cardiac glycosides.

c. Baljet test: To the substance sodium picrate solution is added. A yellow to orange colour indicates the presence of cardiac glycosides.

5. Test for Proteins and free amino acids

a. Millon's Test: A small quantity of acidulous-methanolic extract of the powdered drug was heated with Millon's reagent. A white precipitate turning red on heating indicates the presence of proteins.

b. Biuret Test: To the methanolic extract of powdered drug, one ml of dilute sodium hydroxide(10%) solution was added followed by this one drop of very dilute copper sulphate solution was added. A violet colour indicates the presence of proteins.

c. Ninhydrin test: To the methanolic extract of powdered drug, two drops of ninhudrin solution (10mg of ninhydrin in 200mL of acetone) were added to 2mL of aqueous filtrate. A characteristic purple colour indicated the presence of amino acid.

6. Test for Mucilage

A few mL of aqueous extract was prepared from the powdered drug was treated with ruthenium red. Pinkish red colour indicates the presence of mucilage.

7. Test for Flavonoids

a. Magnesium turning- con HCl test: A little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added and Boiled for five minutes. A red colour indicates the presence of flavonoids.

b. Alkali Test: To the small quantity of test solution 10% aqueous sodium hydroxide Solution was added. A yellow orange color indicates the presence of flavonoids.

c. Acid Test: To a small quantity of test solution, few drops of concentrated sulphuric acid was added. A yellow orange color indicates the presence of flavonols.

8. Test for terpenoids

The powdered leaf was shaken with petroleum ether and filtered. The filtrate was evaporated and residue obtained was dissolved in small amount of chloroform and the chloroform solution tin and thionyl chloride were added. A pink color indicates the presence of terpenoids.

9. Test for phenolic compounds

Small quantity of powdered sample was tested with the following reagents and the colour produced indicates the presence of phenolic compounds.

a. 5% Ferric chloride solution --- Deep bluish black colour.

b. Lead acetate solution --- White precipitate.

c. Bromine water --- Decolouration of bromine water.

d. Acetic acid solution --- Red colour.

e. Dilute iodine solution --- Transient red colour.

f. Tannic acid --- Precipitate.

10. Test for Tannins

A small quantity of the powdered drug was extracted with water. To the aqueous extract, few drops of ferric chloride solution were added. A bluish black color indicates the presence of tannins.

11. Test for saponins

About 0.5g of the powdered drug was boiled gently for 2min with 20mL of water and filtered while hot and allowed to cool. 5mL of the filtrate was then diluted with water and shaken vigorously. A frothing indicates the presence of saponins.

12. Test for volatile oil

About 250gm of fresh leaves of *Citrullus lanatus* was weighed and subjected to hydrodistillation separately using volatile oil estimation apparatus (BP 1980). Absence of volatile oil.

The above chemical tests were carried out on aqueous and methanolic extract of *Citrullus lanatus* leaf powder and the results were tabulated in **Table 5.**

SECTION B - QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Phytochemical constituents such as tannins, flavonoids, phenols, alkaloids and several other aromatic compounds or secondary metabolites of plants serve as a defence mechanism against predation by many microorganism, insects and herbivores. The curative properties of medicinal plants are perhaps due to the presence of these secondary metabolites Medicinal plants may be used to cure some common and other various diseases.[91]

Estimation of total phenol content [92-93]

Natural bioactive compounds like phenols and flavonoids are important secondary metabolites in plants having intrinsic properties that affect appearance, taste, odour and oxidative stability of plant based foods. These compounds also posses biological properties like antioxidant, anti-aging, anti-carcinogen, protection from cardiovascular, immune and autoimmune diseases and brain dysfunctions viz. Parkinson's, Alzheimer's, Huntington's diseases, etc

Principle [94-98]

The total phenolic content of methanolic extracts of *Citrullus lanatus* were determined by Folin Ciocalteu reagent method. All the phenolic compounds are oxidized by the Folin-Ciocalteu Reagent and the reaction was neutralized with sodium carbonate, which is reduced during oxidation of the phenolic substances, into a mixture of blue molybdenum and tungsten oxides. The blue colour produced has a maximum absorption at about 760nm. The absorption is proportional to the quantity of oxidized phenolic compounds. The absorbance of the resulting blue colour was measured at 760nm, using gallic acid as standard.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

a) Folin Ciocalteu Reagent (1N)

Commercially available Folin Ciocalteu Reagent (2N) was diluted with an equal volume of distilled water. The resultant solution was kept in a brown color bottle and stored in refrigerator at 4ºC.

b) Sodium carbonate solution (10%)

Procedure

Gallic acid was accurately weighed and diluted in water to concentration of 1mg/mL. This solution was suitably diluted to get concentrations ranging from 2, 4, 6, 8, 10µg/mL. 0.5mL of Folin Ciocalteu reagent was added and allowed to stand for 15min. Then 1mL of 10% sodium carbonate solution was added. Finally the mixtures were mixed with distilled water and made upto10mL, allowed to stand for 30min at room temperature and total phenols were determined by spectrophotometrically at 760nm using the reagent as blank.

The methanolic extract of *Citrullus lanatus* was weighed and diluted to get a solution of 1mg/mL. Different concentrations of the solution were taken in separate test tubes. 0.5mL of Folin Ciocalteu reagent was added and allowed to stand for 15min. Then 1mL of 10% sodium carbonate solution was added. Finally the mixtures were mixed with distilled water and made upto10mL, allowed to stand for 30min at room temperature and total phenols were determined by spectrophotometrically at 760nm using the reagent as blank.

A calibration curve was generated by plotting concentration of gallic acid versus absorbance **(Fig.9)**. A linear regression equation was determined using regression analysis. The total phenol content was calculated using the linear regression equation and expressed in terms mg of gallic acid equivalent per gm of extract (mg GAE/g). The results obtained are presented in **Table 6.**

Estimation of total flavonoids content [99-101]

Principle

The aluminum chloride colorimetric technique was used for estimation of total flavonoid estimation .Aluminum ions form stable complexes with C4 keto group and either to C3 or C5 hydroxyl groups of flavones and flavonols in acidic medium. It also forms acid labile complexes with ortho hydroxyl groups in the A or B rings of flavonoids. These

complexes showed a strong absorption at 415nm which is used for the estimation of flavonoids.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

10% aluminum chloride

1M potassium acetate

Procedure

An aliquot quantity of quercetin was dissolved in ethanol to get a stock solution of 1mg/mL. Further dilutions were made to get concentrations ranging from 20-100µg/mL. 1mL of the above standard solutions were taken in different volumetric flasks, 0.1mL of aluminum chloride solution, 0.1mL of potassium acetate solution and 2.8mL of ethanol were added and the final volume was then made up to 5mL with distilled water. After 20min the absorbance was measured at 415nm. A sample without aluminium chloride was used as a blank. From the absorbance obtained, a calibration curve was constructed by plotting concentration versus absorbance of quercetin (**Fig**.**10)**. 1mL of methanolic extract at concentrations 50µg/mL and 100µg/mL were taken and the reaction was carried out as above and the absorbance was measured at 415nm after 20min and the readings were tabulated in **Table 7.** The amount of flavonoids present can be determined by linear regression analysis. The total flavonoid content was expressed as mg querecetin equivalents /g of extract.

Estimation of total tannin content [102-103]

Tannins are naturally occurring polyphenolic compounds of varying structure. Tannins are having antioxidant and microbial activities and also used as antiseptic and astringents. They are divided into two main groups namely hydrolysable and condensed.

Hydrolysable and tannins contain a polyhydric alcohol and condensed tannins are mostly flavonols.

Principle

The tannins are estimated by Folin-Denis Method. This is based on the non stoichiometric oxidation of the molecules containing a phenolic hydroxyl group. Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce a highly colored blue solution. The intensity is directly proportional to the amount of tannins and measured in a spectrophotometer at 700nm.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

Folin-Denis reagent

10% Sodium carbonate solution

Procedure

About 0.2mL of methanolic extract of *C. lanatus* was pipetted into test tubes. To this, 0.5ml of Folin-Denis reagent and 0.8mL of distilled water was added. The tubes were kept aside for 15min. To this, 1mL of sodium carbonate solution was added and the remaining volume was made up with 7.5mL of distilled water. Then the tubes were shaken and the absorbance was recorded at 700nm after 30min. Tannic acid, used as a standard was taken at different concentration i.e 2, 4, 8, 12, 16, 20µg/mL in different test tubes and the procedure adopted above was followed. The calibration curve for tannic acid was plotted using concentration versus absorbance (**Fig.11).** A linear regression equation was calculated and the equation was used to calculate the amount of total tannins as tannic acid equivalent. The amount of tannin content is expressed in mg/g of extract. The results obtained are presented in **table 8.**

Estimation of vitamins[104-108]

Nutrients are building blocks of the human body that regulate essential body functions as well as furnish them with the energy for their work. Nutrients are divided into macro-nutrients (proteins, fats, carbohydrates) and micronutrients (vitamins and minerals).

Vitamins are class of micro nutrients which play an essential role in human health and are classified into water soluble (niacin, riboflavin, and thiamine) and fat soluble (retinol and tocopherol). Plants synthesize most of the vitamins and serve as primary sources of these dietary essentials.

Riboflavin is involved in the regulatory functions of some hormones that are connected with carbohydrate metabolism.

Niacin (Vitamin B3) is essential for the normal functioning of the skin, intestinal tract and the nervous system.

Tocopherol as a lipophilic vitamin is the most powerful antioxidant. Tocopherol protects the red blood cell from hemolysis, boosts the immune response, and reduces the risk of myocardial infarction by reducing the oxidation of LDL as well as acting as an anti-mutagen. It also functions synergistically with other antioxidants like vitamin A and C and selenium.

Vitamin C functions as a water soluble antioxidant.

Carotenoids are antioxidant compounds found in plants that can enhance the human health immune response by playing preventive roles against degenerative diseases such as: cancer, carcinogenesis and cardiovascular diseases, vision related abnormalities, Parkinsonism, infertility, etc. This source of **vitamin A** from vegetables and fruits is the main

source for people living in developing countries and makes up about 70-90% of their dietary Vitamin A intake.

The composition of the water-insoluble vitamins, riboflavin and thiamine were determined by the method of Okwu, D.E and C.Joshi, and ascorbic acid content was determined by the method of *Sarojini et al*.

Estimation of vitamin B_1 [109-112]

Principle

The estimation of Vitamin B_1 was carried out using the method of Okwu, D.E and C. Joshi with slight modification. The reaction of thiamine hydrochloride with potassium dichromate ($K_2Cr_2O_7$) has been studied in alcoholic solution at room temperature. The compound obtained is ionic 1:1 in which thiamine is present as a cation and $Cr_2O_7^{2-}$ as an anion. The chemical formula of the salt, $(C_{12}H_{18}N_4OS)$ (Cr_2O_7) (symbolically $ThHCr_2O_7$), has been established by UV/Vis spectra absorbance of about 360nm.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagent

0.01% potassium dichromate solution

Sample Preparation

10g of powdered plant material was homogenized with ethanolic sodium hydroxide (100mlL. The plant extract was filtered and used as a sample.

Procedure

Thiamine was weighed and dissolved in water to get stock solution of 2mg/mL. Further dilutions were made to get the concentrations ranging from 100-500µg/mL. To 10mL

of sample, 10mL of potassium dichromate solution was added and the colour produced was measured at 360nm. A calibration curve was constructed by plotting concentration versus absorbance of thiamine **(Fig.12).** The above procedure was repeated for the plant extract and the absorbance was measured at 360nm and the readings were tabulated in **Table 9.** The amount of vitamin B_1 present can be determined by linear regression analysis. The vitamin B_1 content was expressed as mg/g of extract.

Estimation of vitamin B_2

Principle

The estimation of vitamin B_2 was carried out using the method of Okwu, D.E and C.Joshi with slight modification. Riboflavin was treated with potassium permanganate ($KMnO_4$) and hydrogen peroxide (H_2O_2). Mixing of hydrogen peroxide solution, whereupon the permanganate colour is destroyed, excess oxygen is expelled and then sodium sulphate was added and an yellow colour was obtained. The absorbance of the colour was measured at 550nm by UV/Vis spectrophotometer.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagent

0.5% potassium permanganate.

30% hydrogen peroxide

40% sodium sulphate

Sample Preparation

10g of powdered plant material was extracted with 50% ethanol solution and shaken for 1h. The plant extract was filtered and used as a sample.

Procedure

Riboflavin was weighed and dissolved in water to get stock solution of 20mg/mL. Further dilutions were made to get the concentrations ranging from 2-10mg/mL. To 15mL of sample and 10mL of 0.5% potassium permanganate and 1mL of 30% hydrogen peroxide were added and allowed to stand over a hot water bath for about 30min. 2mL of 40% sodium sulphate was added. This was made up to 5mL. The absorbance of the chromogen was measured at 550nm in a UV visible spectrophotometer. A calibration curve was constructed by plotting concentration versus absorbance of riboflavin **(Fig. 13).** The above procedure was repeated for the plant extract and the absorbance was measured at 550nm and the readings were tabulated in **Table 10.** The amount of vitamin B_2 present can be determined by linear regression analysis. The vitamin B_1 content was expressed as mg/g of extract.

Estimation of vitamin C [113-114]

Principle

The estimation of vitamin C was carried out using the method of *Sarojini et al.,* with slight modifications. The keto group of ascorbic acid undergoes a condensation reaction with 2,4 dinitro phenyl hydrazine to form a hydrazone which is orange yellow and has an absorbance of about 520nm.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

0.2% dinitro phenyl hydrazine

85% sulphuric acid

Sample Preparation

Methanolic extract of *Citrullus lanatus* plant extract was and used as a sample.

Procedure

Ascorbic acid was weighed and dissolved in water to get stock solution of 1mg/mL. Further dilutions were made to get the concentrations ranging from 40-200µg/mL. To 1mL of sample 0.5mL of di nitro phenyl hydrazine solution was added and incubated for 3h at 37ºC. After 3h, 2.5mL of 85% sulphuric acid was added and the absorbance was measured after 30min at 520nm. A calibration curve was constructed by plotting concentration versus absorbance of ascorbic acid **(Fig.14).** The procedure was repeated for the plant extract as above and the absorbance was measured at 520nm after 3h and the readings were tabulated in **Table 11.** The amount of vitamin C present can be determined by linear regression analysis. The vitamin C content was expressed as mg/g of extract.

SECTION C - CHROMATOGRAPHY

Chromatography is a non-destructive procedure for resolving a complex mixture into its individual fractions or compounds. "Chromato" "graphy" derives its name from two words as chromo= colour and graphy= writing. i.e colour bands are formed in the procedure which are measured or analysed. It is defined as the process of separation of the individual components of a mixture based on their relative affinities towards stationary and mobile phases. These two phases can be can be solid-liquid, liquid-liquid or gas-liquid.

Principle

The samples are subjected to flow by mobile liquid onto or through the stable stationary phase. The sample components are separated into fractions based on their relative affinity towards the two phases during their travel. The fraction with greater affinity to stationary layer travels slower and shorter distance while that with less affinity travels faster and longer. Overall available **chromatography techniques** for regular analysis include,

a) Column chromatography.

b) High performance liquid chromatography.

c) Gas chromatography.

d) Ion-exchange chromatography.

e) Size exclusion chromatography.

f) Thin layer chromatography.

g) High performance thin layer liquid chromatography.

h) Paper chromatography.

i) Affinity chromatography.

THIN LAYER CHROMATOGRAPHY[115]

The term "thin-layer chromatography", introduced by E. Stahl in 1956, means a chromatographic separation process in which the stationary phase consists of a thin layer applied to a solid substrate or "support". Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) – now also called planar chromatography.

Thin-layer chromatography or TLC, is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents. TLC is a quick, inexpensive microscale technique that can be used to:

• determine the number of components in a mixture

• verify a substance's identity

• monitor the progress of a reaction

• determine appropriate conditions for column chromatography.

• analyze the fractions obtained from column chromatography.

Principle

TLC is based on the principle of adsorption. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) travel over the surface of stationary phase and during this movement, the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved. [122-126]

Preparation of TLC Plates

The adsorbent (silica gel G) slurry was prepared in water in the ratio of (1: 2). The glass plates (20cm x 5cm) were cleaned and laid in a row as a template, the suspension was poured into Stahl TLC spreader, which was adjusted to 0.25mm thickness and coated in a single passage of the spreader over them. These plates were air dried and activated in hot air oven at 105ºC for 30min and kept in a dessicator. The plates were used as the stationary phase or Pre-coated aluminum plates coated with silica gel G F_{254} (Merck) were also used for analysis.

Sample application

The extracts were dissolved in mobile phase and the spot was applied on the TLC plates using capillary tube.

Development of the chromatogram

After drying of the spot, the plates were developed in a chromatographic tank containing the solvent system. After one third of the plate was developed the plates were taken outside and dried. The TLC plate was examined visually or under UV light

Solvent system I

Stationary phase - Silica gel G

Mobile phase - Toluene : Ethyl Acetate : Methanol (7:2:1)

Detecting agent - visual & UV light

Solvent system II

Stationary phase - Silica gel G

Mobile phase - Chloroform : Methanol (9.5:1)

Detecting agent - visual & UV light

The R_f values were calculated using the formula [Distance travelled by solute/ Distance travelled by solvent]. The phytochemical evaluation of methanolic extract of *Citrullus lanatus* was carried out by using TLC studies. The results are presented in **Table 12** and **Fig 15 & 16.**

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

High performance thin layer chromatography (**HPTLC**) is an enhanced form of thin layer chromatography (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements. [116]

Analysis of pharmaceutical and natural compounds and newer drugs is commonly used in all the stages of drug discovery and development process. High-performance thin layer chromatography is one of the sophisticated instrumental techniques based on the full capabilities of thin layer chromatography. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, and so on enable it to be a powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, food stuffs, and so on. [117]

Principle

HPTLC take place in high speed capillary flow range of the mobile phase. There are three main steps in HPTLC

1] sample to analyzed to chromatogram layer volume precision and suitable position are achieved by use of suitable instrument.

2]solvent (mobile phase) migrates the planned distance in layer(stationary) by capillary action in this process sample separated in its components.

3] separation tracks are scanned in densitometer with light beam in visible or uv region.[118]

The phytochemical evaluation of methanolic extract of *Citrullus lanatus* was carried out by using HPTLC studies. The results are presented in **Table 13**and **Fig. 17** to **20.**

HPTLC fingerprint Development

Instrument used

CAMAG TLC Scanner 3 "Scanner3-070408"S/N 070408(1.41.21) was used for detection and CAMAG Linomat 5 sample applicator was used for the application of the track. Twin trough plate development chamber was used for development of chromatogram. Software used was winCATS 1.4.3

Sample

The Methanolic extract of *Citrullus lanatus* was dissolved in mobile phase and 2µl sample is applied as 8mm band was used for taking HPTLC fingerprint.

Stationary Phase

HPTLC plates silica gel 60 F 254

Mobile phase

Toluene: ethyl acetate: methanol (7: 2: 1) was used as the mobile phase for development of chromatogram. The mobile phase was taken in a CAMAG twin trough glass chamber.

Detection wavelength

The developed plates were examined at wavelength 254 and 366nm and @520nm in Densitometry TLC Scanner 3. The TLC visualization, 3D display of the finger print profile and peak display at 254 and 366nm are presented in **Table 13** and **Fig 17** to **20.**

RESULTS AND DISCUSSION

SECTION A - PRELIMINARY PHYTOCHEMICAL SCREENING

The results obtained for the preliminary phytochemical screening of the aqueous and methanolic extract of *citrullus lanatus* leaf was presented in **Table 5.**

Table. 5: Preliminary phytochemical screening for the aqueous and methanolic extract of *Citrullus lanatus* leaf

S. No.	TEST	RESULTS	
		Aqueous extract	Methanolic extract
1.	**TEST FOR CARBOHYDRATES**		
	a. Molisch's test	+	+
	c. Benedict's test	+	+
	b. Fehling's test	+	+
2.	**TEST FOR ALKALOIDS**		
	a. Mayer's reagent	+	+
	b. Dragendroff's reagent	+	+
	c. Hager's reagent	+	+
	d. Wagner's reagent	+	+
3.	**TEST FOR PHYTOSTEROLS**		
	a. Salkowski's test	+	+
	b. Libermann- burchard's test	+	+
4.	**TEST FOR GLYCOSIDES**		
	a. Anthraquinone glycosides	-	-
	i) Borntrager's test	-	-
	ii) Modified Borntrager's test	-	-
	b. Cardiac glycosides		
	i) Keller Killiani test	+	+
5.	**TEST FOR PROTEINS**		
	a. Millon's test	+	+
	b. Biuret test	+	+
	AMINO ACIDS		
	a. Ninhydrin test	+	+
6.	**TEST FOR MUCILAGE**	-	-

7.	**TEST FOR FLAVONOIDS**		
	a. Shinoda test	+	+
	b. Alkali test	+	+
	c. Acid test	+	+
8.	**TEST FOR TERPENOIDS**	+	+
9.	**TEST FOR PHENOLIC COMPOUNDS**		
	a. 5% Ferric chloride solution	+	+
	b. Lead acetate solution	+	+
	c. Bromine water	+	+
	d. Acetic acid solution	+	+
	e. Dilute iodine solution	+	+
	f. Tannic acid	+	+
10.	**TEST FOR TANNINS**		
	$FeCl_3$ test	+	+
11.	**TEST FOR SAPONINS**		
	Foam test	+	+
12.	**TEST FOR VOLATILE OILS**	**-**	**-**

(+) indicates positive reaction (-) indicate negative reaction

The preliminary phytochemical screening procedure of the aqueous and methanolic extract of *Citrullus lanatus* leaf showed the presence of carbohydrates, alkaloids, phytosterols, cardiac glycoside, protein and amino acids, flavanoids, terpenoids, phenolic compounds, tannins, saponins and volatile oil were absent.

SECTION B - QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Estimation of total phenols

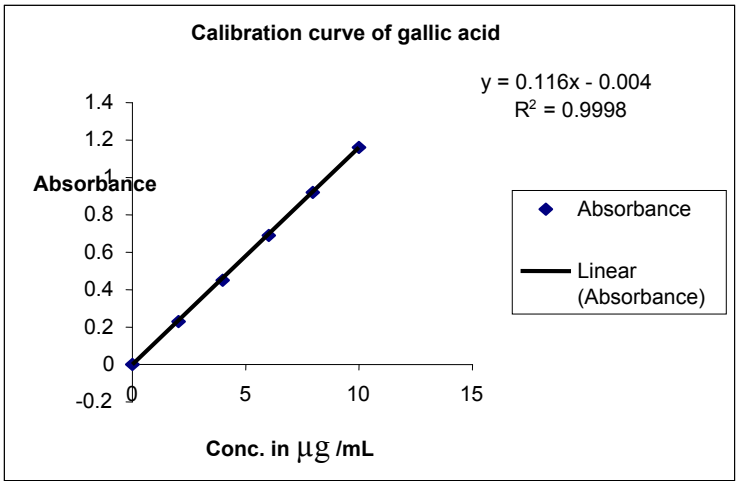
The results for the total phenol estimation of methanolic extract of *Citrullus lanatus* are tabulated in **Table 6** and **Fig.9.**

Table 6: Total phenolic content in ethanolic extract of *Citrullus lanatus* in terms of gallic acid equivalents

S. No.	Conc. of gallic acid in µg/mL	Absorbance at 760nm	Conc. of extract in µg/mL	Abs at 760nm*	Amount of total phenolic content in terms mgGAE/g of extract*
1	2	0.229 ± 0.010	50	0.256±0.004	43.90±0.304
2	4	0.452 ± 0.006	100	0.578±0.004	50.20±0.373
3	6	0.695 ± 0.005			
4	8	0.918 ± 0.031			
5	10	1.162 ± 0.028		**Average**	**47.05±0.338**

*** mean of three readings ±SEM**

Fig . 9: Calibration curve of Gallic acid



The linear regression equation was found to be y=0.116x-0.004 while the correlation coefficient was found to be 0.9998. The amount of phenol content present in the extract in terms mg GAE/g of extract was found to be **47.05 ± 0.338** by using the above linear regression equation.

Estimation of total flavonoids

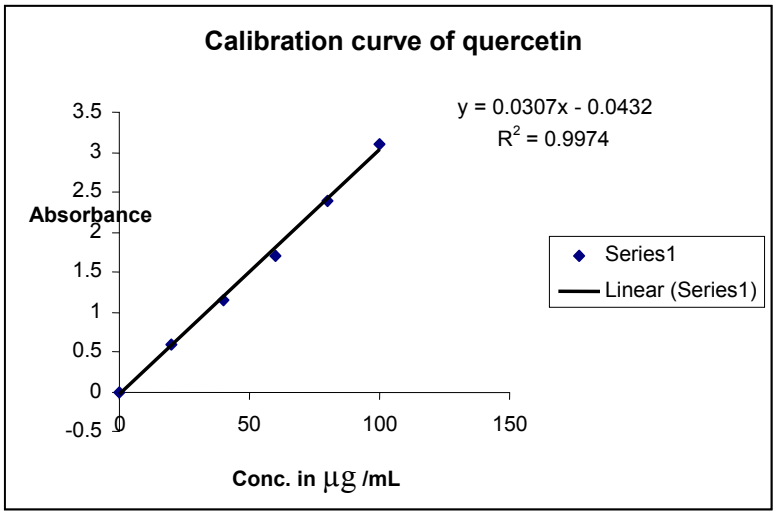
The results for the total flavonoid estimation of methanolic extract of *Citrullus lanatus* are tabulated in **Table 7 .**

Table 7: Total flavonoid content per gram of extract in terms of quercetin by aluminium chloride method

S. No.	Conc. of quercetin in µg/mL	Absorbance at 415nm	Conc. of methanolic extract in µg/mL	Absorbance at 415nm	Amount of total flavonoid content in terms mg quercetin equivalent/ g of extract
1	20	0.589 ± 0.01	50	0.090±0.001	86.55±0.21
2	40	1.151 ± 0.04	100	0.243±0.003	93.44±0.39
3	60	1.710 ± 0.09			
4	80	2.390 ± 0.03			
5	100	3.112 ± 0.03		**Average**	**89.99±0.30**

***mean of three readings** ± SEM

Fig. 10 : Calibration curve of quercetin



The linear regression equation was found to be y=0.0307x-0.0432 while the correlation was found to be 0.9974. The amount of flavonoid content present in the extract in terms mg quercetin equivalent/g of extract was found to be **89.99 ± 0.30** by using the above linear regression equation.

Total tannin estimation [12]

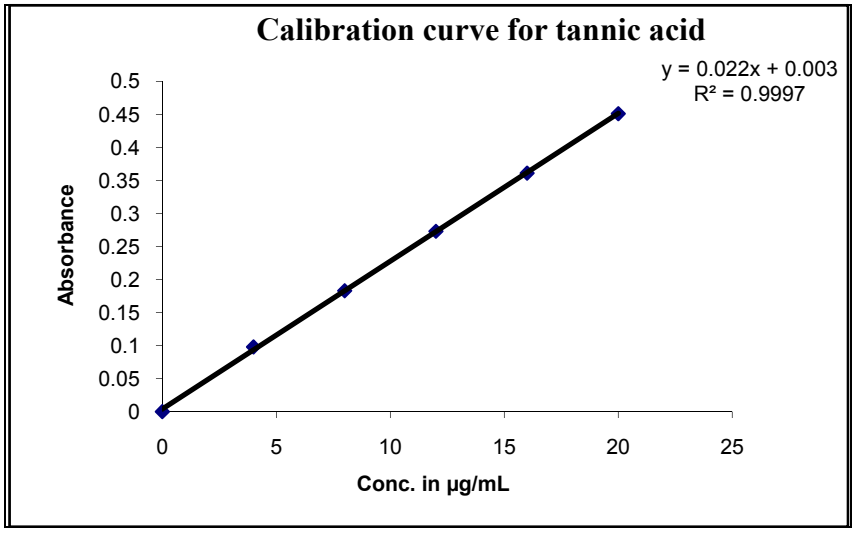
The results for the total tannin estimation of methanolic extract of *Citrullus lanatus* are tabulated in **Table 8.**

Table 8: Total tannin content in ethanolic extract of *Citrullus lanatus* in terms of tannic acid equivalents

S. No.	Conc. of tannic acid in µg/mL	Absorbance at 760nm	Conc. of methanolic extract in µg/mL	Absorbance at 760nm*	Amount of total tannin content in terms mg tannic acid/g of extract*
1	4	0.098 ± 0.020	10	0.060±0.03	260.60±1.51
2	8	0.183 ± 0.010	20	0.131±0.07	292.42±2.00
3	12	0.203 ± 0.010			
4	16	0.361 ± 0.200			
5	20	0.451 ± 0.100		**Average**	**276.51±1.75**

*** mean of three readings ±SEM**

Fig. 11 : Calibration curve of tannic acid



Total tannin determination is carried out by spectrophotometry after oxidation of the analyte with the Folin–Denis reagent in alkaline medium. This method is based on a redox reaction and other reducing agents in the samples.

The linear regression equation was found to be y = 0.022x + 0.003 while the correlation was found to be 0.9997. The amount of tannin content present in the methanolic extract of *Citrullus lanatus* in terms of mg tannic acid/g of extract was found to be **290.9 ± 0.12** by using the above linear regression equation.

Vitamic B_1 Content

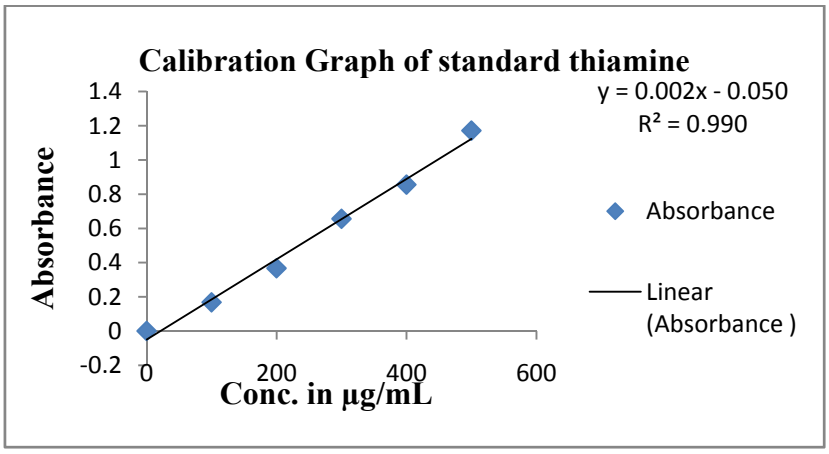
The results for vitamin B_1 content of methanolic extract of *Citrullus lanatus* are presented in **Table 9.**

Table 9: Estimation of Vitamin B_1 in *Citrullus lanatus*

S. No.	Conc. of Thiamine in µg/mL	Absorbance at 360nm	Conc. of methanolic extract in µg/mL	Absorbance at 360nm	Amt of vitamin B_1 present mg/ g of extract
1	100	0.168 ± 0.001	100	0.286 ± 0.004	54.82 ± 0.006
2	200	0.367 ± 0.002	200	0.321 ± 0.007	55.02 ± 0.006
3	300	0.656 ± 0.002	300	0.456 ± 0.003	59.00 ± 0.002
4	400	0.856 ± 0.001		**Average**	**56.28 ± 0.004**
5	500	1.172 ± 0.003			

***mean of three readings** ± SEM

Fig. 12: Calibration curve of thiamine



The linear regression equation was found to be y = 0.0023x - 0.0501 and a correlation coefficient of 0.990. The amount of vitamin B_1 content present in the methanolic extract of *Citrullus lanatus* was found to be 56.28 ± 0.004 mg/gm by using the above linear regression equation.

Vitamic B_2 Content

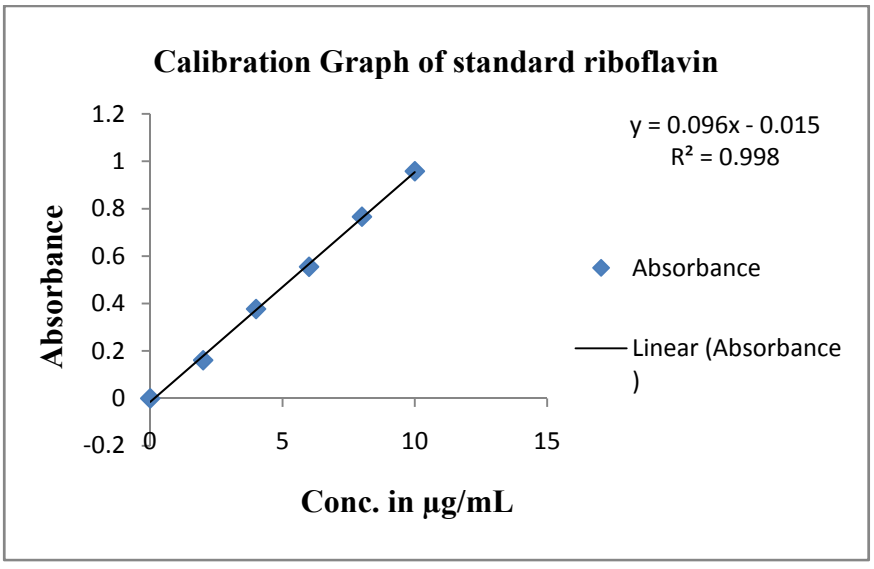
The results for vitamin B_2 content of methanolic extract of *Citrullus lanatus* are presented in **Table 10.**

Table 10: Estimation of Vitamin B_2 in *Citrullus lanatus*

S. No.	Conc. of Riboflavin in mg/mL	Absorbance at 360nm	Conc. of methanolic ext. in mg/mL	Absorbance at 360nm	Amt of vitamin B_2 present mg/ g of extract
1	2	0.161 ± 0.006	2	0.047 ± 0.004	31.99 ± 0.011
2	4	0.377 ± 0.012	4	0.111 ± 0.007	32.50 ± 0.006
3	6	0.555 ± 0.002	6	0.203 ± 0.003	37.29 ± 0.010
4	8	0.766 ± 0.005			
5	10	0.958 ± 0.004			
				Average	**34.00 ± 0.009**

***mean of three readings** ± SEM

Fig. 13: Calibration curve of riboflavin



The linear regression equation was found to be y = 0.0969x - 0.015 and a correlation coefficient of 0.9989. The amount of vitamin B_2 content present in the methanolic extract of *Citrullus lanatus* was found to be 34.00 ± 0.009 mg/gm by using the above linear regression equation.

Vitamic C Content

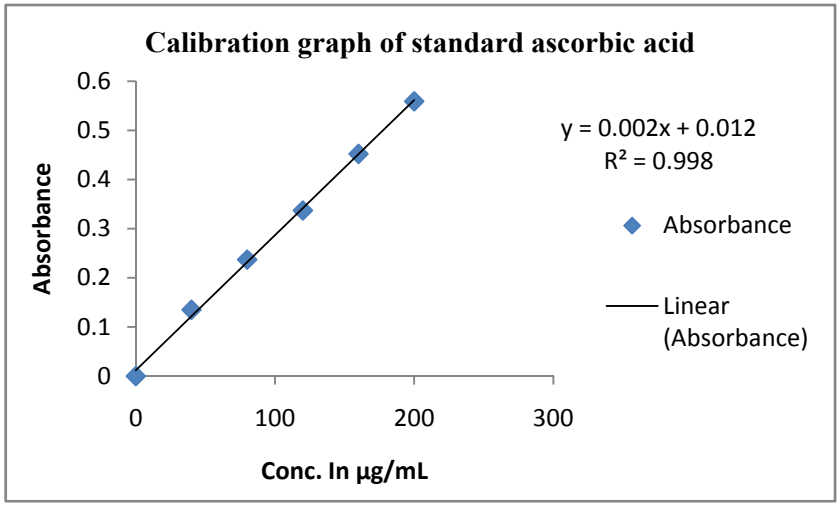
The results for vitamin C content of methanolic extract of *Citrullus lanatus* are presented in **Table 11.**

Table 11 : Estimation of Vitamin C in *Citrullus lanatus*

S. No.	Conc. of ascorbic acid in µg/mL	Absorbance at 520nm	Conc. of methanolic ext in µg/mL	Absorbance at 520nm	Amt of vitamin C present / g of extract
1	40	0.135 ± 0.000	100	0.076 ± 0.004	237.03 ± 0.006
2	80	0.265 ± 0.015	200	0.137 ± 0.007	253.70 ± 0.006
3	120	0.346 ± 0.010			
4	160	0.468 ± 0.011			
5	200	0.525 ± 0.010			
				Average	**245.37 ± 0.006**

***mean of three readings** ± SEM

Fig.14 : Calibration curve of ascorbic acid



The linear regression equation was found to be y = 0.0027x + 0.012 and a correlation coefficient of 0.9982. The amount of vitamin C content present in the methanolic extract of *Citrullus lanatus* was found to be 245.37 ± 0.06mg/gm by using the above linear regression equation.

SECTION C – CHROMATOGRAPHY

THIN LAYER CHROMATOGRAPHY

The number of spots, Rf value of the same and the colour of the spots under UV light 366nm and visible light is presented in **Table 12** and the photograph of the plate is presented in **Figs. 15 & 16.**

Table 12: Phytochemical evaluation of methanolic extract of *Citrullus lanatus* by TLC studies

S. No	Solvent system	Detecting agent	No. of spots	Colour of spots	R_f values
1.	Toluene: Ethyl acetate : Methanol (7 : 2 :1)	Under UV at 366nm	5	Orange	0.94
				Dark orange	0.84
				Dark red	0.72
				Light pink	0.45
				Light pink	0.37
		Under Visible light	6	Yellow	0.92
				Brown	0.82
				Dark green	0.72
				Yellowish green	0.58
				Yellowish green	0.47
				Yellow	0.35
2	Chloroform: Methanol (9.5 : 1)	Under UV at 366nm	4	Dark brown	0.90
				Dark orange	0.72
				Yellow	0.68
				Light pink	0.46
		Under Visible light	6	Dark green	0.92
				Yellow	0.74
				Yellow	0.70
				Light green	0.52
				Brown	0.46
				Brown	0.34

The extract showed 5 spots at 366nm and 6 spots at visible light. The R_f value of 0.72, 0.37 and 0.45 may be due to the presence of flavonoids, phenolic compounds and tannin. When viewed under UV at 366m and visible light after development in the mobile phase namely chloroform: methanol (9.5:1). The Rf value of 0.9 and 0.72 may be due to the presence of Cucurbitacin glucoside B and Cucurbitacin glucoside E. The extract also showed

SECTION C - CHROMATOGRAPHY

THIN LAYER CHROMATOGRAPHY

Fig. 15 : TLC analysis of methanolic extract of *Citrullus lanatus* @ 366nm and Visible light

Mobile phase - Toluene : Ethyl acetate : Methanol (7: 2 : 1)

@ 366nm

@ Visible light

Fig. 16 : TLC analysis of methanolic extract of *Citrullus lanatus* @ 366nm and Visible light

Mobile Phase : Chloroform : Methanol (9.5 : 1)

@ 366nm **@ Visible light**

different R_f value under UV at 366nm and Visible light. The R_f may be due to the presence of different active principle might be responsible for the therapeutic activity.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

The visualization of the HPTLC plate of methanolic extract of *Citrullus lanatus* at 254nm and 366nm is presented in **Fig 17.** The photo of plate at 254nm showed the presence of 8 spots while at 366nm showed the presence 8 spots.

The 3D display of the fingerprint profile and the peak display of methanolic extract of *Citrullus lanatus* at 254nm and 366nm is presented in **Figs 18.** The display at 254nm shows the presence of 8 peaks while at 366nm shows the presence 8 peaks. The R_f values of the peaks along with the area under the curve for each peak at 254 and 366 nm are tabulated in **Table 13.**

Table 13: Rf value of the spots and their area under curve at 254 and 366nm

S. No	R_f Value @ 254nm				AREA (AU) @ 254nm			
	TRACK				TRACK			
	1	2	3	4	1	2	3	4
1	0.38	0.46	0.71	0.15	9798.0	27344.1	1187.6	248.6
2				0.18				156.4
3				0.36				1320.9
4				0.45				17028.3
5				0.65				1513.1
6				0.72				326.7
7				0.77				291.7
8				0.84				2203.4

S. No	@366nm	
	R_f Value	AREA (AU)
	1	2
1	0.15	758.8
2	0.18	135.7
3	0.39	1647.0
4	0.50	1881.2
5	0.65	9744.8
6	0.72	941.5
7	0.80	348.2
8	0.85	2984.6

The HPTLC finger print profile of the methanolic extract of *Citrullus lanatus* Rf values compared with standard quercetin, gallic acid and catechin. The methanolic extract of *Citrullus lanatus* R_f values also coincided with standard R_f values and hence it may be confirmed that the methanolic extract showed the presence of quercetin, gallic acid and catechin.

Fig.17: Visualization of TLC plate

@ 254nm **@366nm**

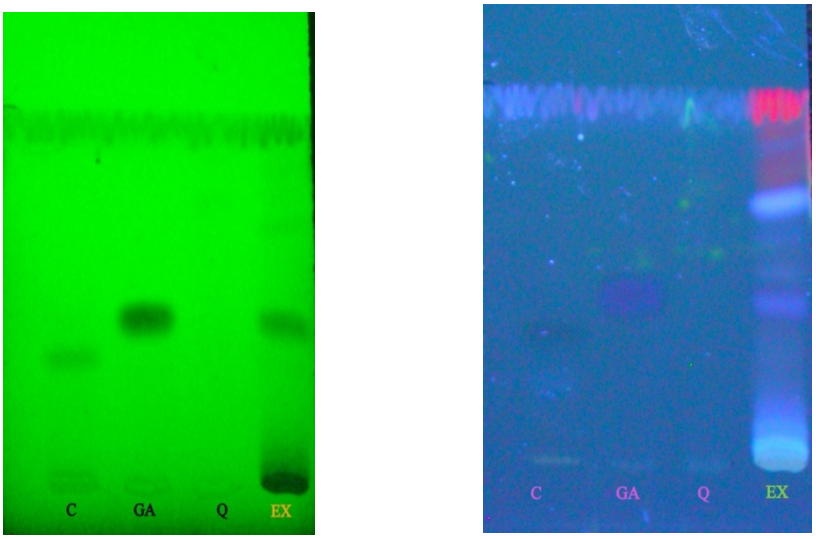


Fig.18: 3D Display of the extract and standards at 254 & 366nm

@ 254nm **@366nm**

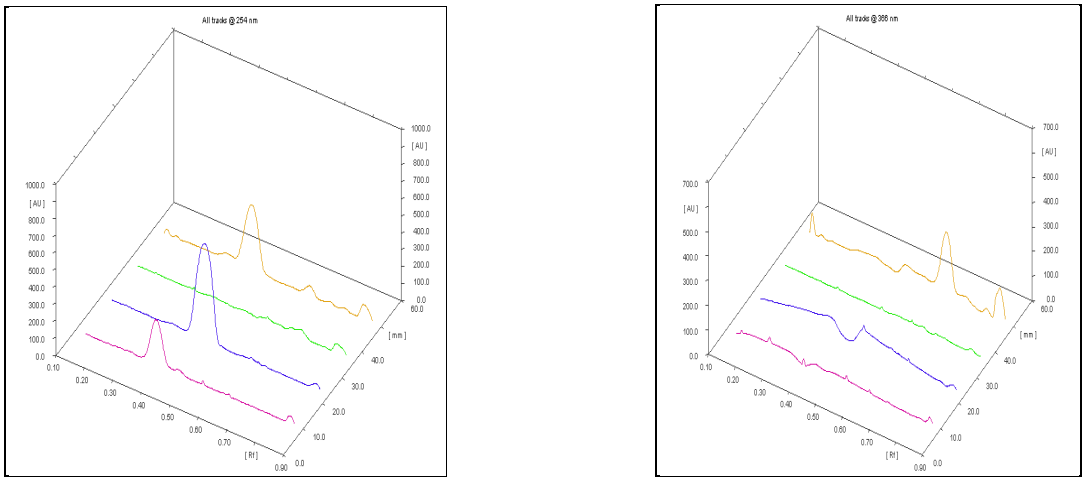
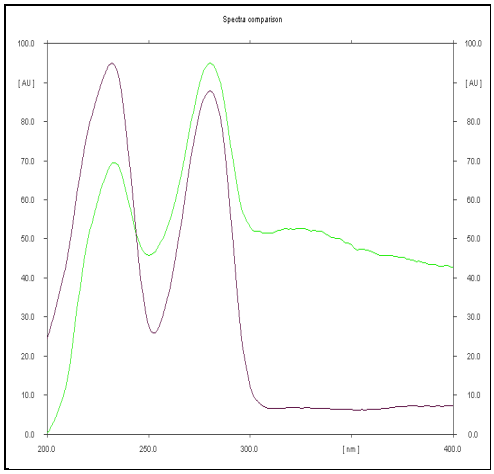
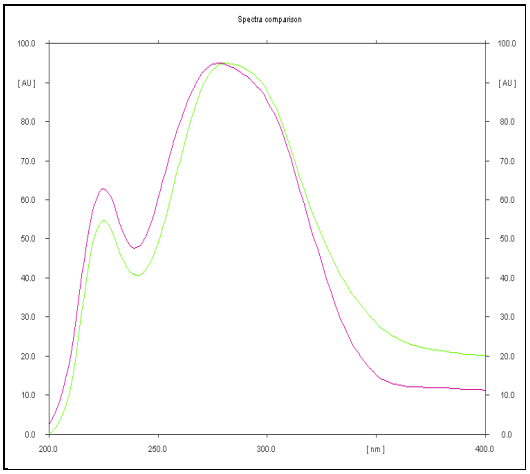


Fig.19: Overlain Spectral Display

For catechin



For gallic acid



For quercetin

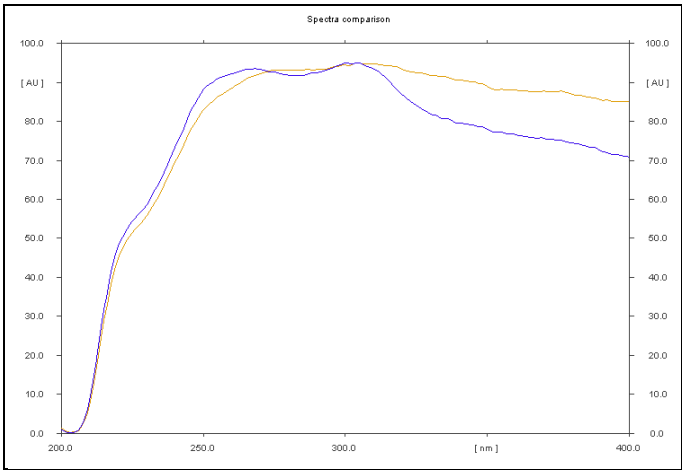
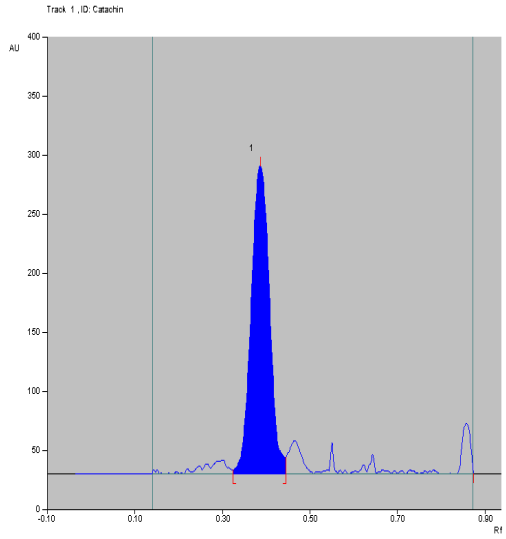


Fig.20: Peak Display

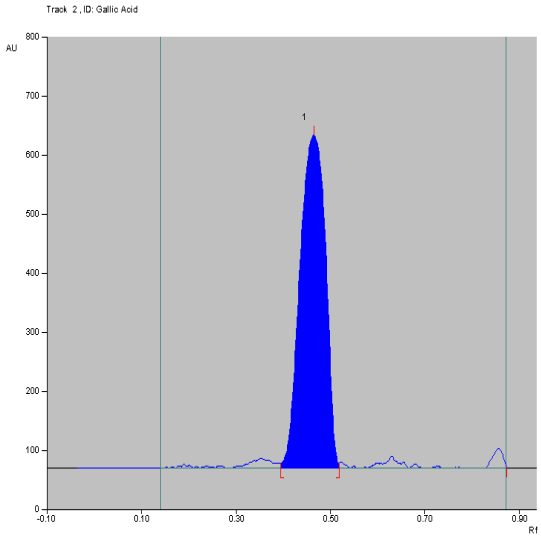
Track 1: Catechin, **Track 2**: Gallic acid,
Track 3: Quercetin, **Track 4**: Methanolic extract of *Citrullus lantus* leaf.

@ 254nm

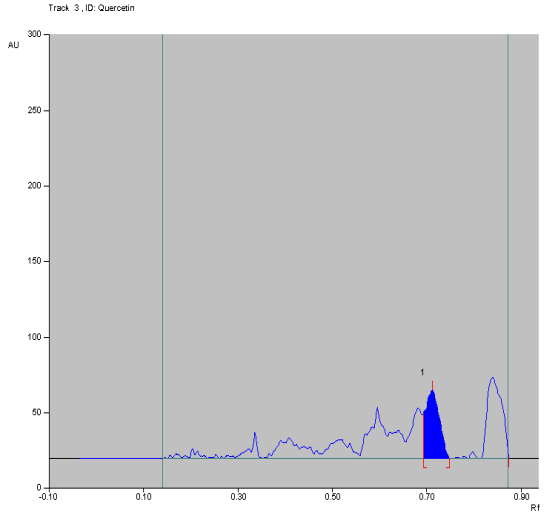
Track 1



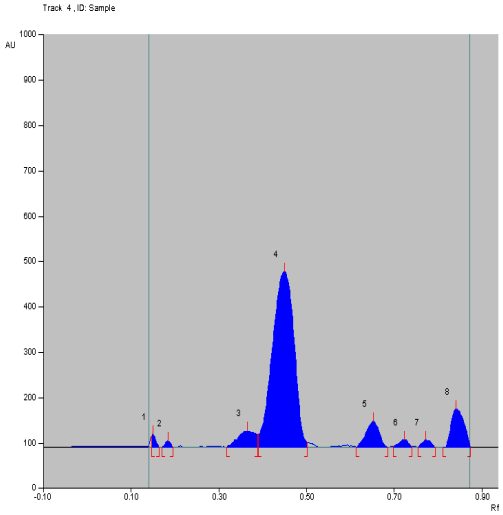
Track 2



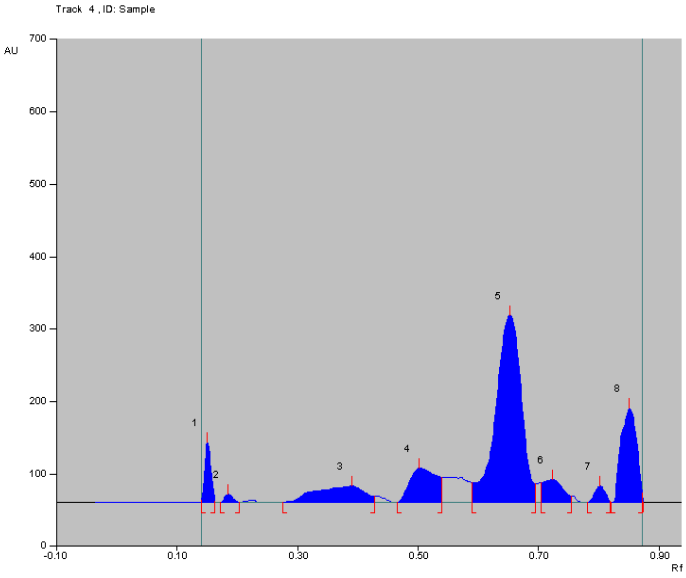
Track 3



Track 4



@366nm
Extract



PHARMACOLOGICAL EVALUATION

CHAPTER VII

PHARMACOLOGICAL EVALUATION[119-120]

The word pharmacology is derived from Greek words pharmacon (an active principle) and logos (a discourse or treatise). It is the science that deals with drugs. It consists of detailed study of drug, particularly their actions on living animal's organ and tissue. The object of pharmacology is mainly to provide such scientific data, using which one can choose a drug treatment of proven efficacy and safety from various options available, so suit the patient.

For thousand of years most drugs were crude natural products of unknown composition and limited efficacy. The overall effect of these substances on the body was rather imprecisely known, but how the same were produced was entirely unknown. The drugs have been purified, chemically characterised and a vast variety of highly potent and selective new drugs have been developed.

SECTION A – *IN VITRO* ANTIOXIDANT ACTIVITY

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by- products of cell metabolism.[121-122].

Free radicals may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species). The oxygen derived molecules are O^{2-} Superoxide], HO [hydroxyl] ,HO_2 [hydroperoxyl], ROO [peroxyl], RO [alkoxyl] as free radical and H_2O_2 oxygen as non-radical. Nitrogen derived oxidant species are mainly NO [nitric oxide], ONOO [peroxy nitrate], NO_2 [nitrogen dioxide] and N_2O_3 [dinitrogen trioxide]. [123-124]

In a normal cell, there is appropriate oxidant: antioxidant balance. However, this balance can be shifted, when production species is increased or when levels of antioxidants are diminished. This stage is called oxidative stress. Oxidative stress results in the damage of biopolymers including nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates.

Oxidative stress causes serious cell damage leading to a variety of human diseases like Alzheimer's disease, Parkinson's disease, atheroscleorosis, cancer, arthritis, immunological incompetence and neurodegenerative disorders, etc. [125-127].

Antioxidants and Health

- Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Diets high in vegetables and fruits, which are good sources of antioxidants, have been found to be healthy; however, research has not shown antioxidant supplements to be beneficial in preventing diseases. Examples of antioxidants include vitamins C and E, selenium, and carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin.
- Rigorous scientific studies involving more than 100,000 people combined have tested whether antioxidant supplements can help prevent chronic diseases, such as cardiovascular diseases, cancer, and cataracts. In most instances, antioxidants did not reduce the risks of developing these diseases.[128]

Medicinal herb as Antioxidants:

Indian medicinal herbs were extensively investigated as vital sources of antioxidants. *Allium cepa* (Onion), *Allium sativum* (Garlic), *Aloe vera* (Indian aloe), *Amomum subulatum* (greater cardamom, Bari elachi), *Andrographis paniculata* (The creat), *Asparagus racemosus* (Shatavari), *Azadirachta indica* (Neem, Nimba), *Bacopa monniera* (Brahmi), *Camellia sinensis* (Green tea), *Cinnamomum verum* (Cinnamon), *Curcuma longa* (Turmeric), *Emblica*

officinalis (Indian gooseberry), *Glycyrrhiza glabra* (Yashtimadhu), *Withania somnifera* (Ashwagandha), and *Zingiber officinalis* (Ginger). [129]

MATERIALS AND METHODS

Method 1: Free radical Scavenging activity using diphenyl picryl hydrazyl (DPPH) free radical

The free radical scavenging activity of the extracts is evaluated by assessing their ability to reduce the colour of DPPH in ethanol according to Brand Williams [130]. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts.

Principle

A simple method that has been developed to determine the antioxidant activity of plants utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 520nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured [130-131].

$$DPPH^{\cdot} + AH \rightarrow DPPH - H + A^{\cdot}$$

Instrument

Shimadzu UV Visible spectrometer, Model 1800

Reagents

0.1mM Diphenyl Picryl Hydrazyl Radical in ethanol

Procedure [132-133]

A stock solution of 0.5mg/mL concentration of methanolic extract of *C. lanatus was* prepared. To 1mL of various concentrations of test samples, 4mL of DPPH was added.

Control was prepared without sample in an identical manner. DPPH was replaced by ethanol in case of blank. The reaction was allowed to be completed in the dark for about 30min. Then the absorbance was measured at 517nm. Vitamin C was used as standard. The percentage scavenging was calculated using the formula [(Control-Test)/Control] x 100. A graph was constructed by plotting concentration versus percentage inhibition and a linear regression equation calculated. The concentration of the sample required for 50% reduction in absorbance (IC_{50}) was calculated using linear regression analysis. A triplicate reading was taken and average was calculated. The results obtained are presented in **Table 14** and **Fig.21.**

Method 2: Total antioxidant activity by Phosphomolybdenum Method. [134-135]

Principle

Total antioxidant capacity was measured by spectrophotometric method of Prieto *et al*. Phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate Mo (V) complex at acidic pH and the absorbance was measured at 695nm. This method is used to determine the total antioxidant activity of samples.

$$Mo^{6+} \rightarrow Mo^{5+}$$

Reagents

0.6M sulphuric acid

28mM sodium phosphate

4mM ammonium molybdate

Instruments

Shimadzu UV Visible spectrophotometer, Model 1800

Procedure

An aliquot of 0.3mL of different concentrations of sample solution was combined with 2.7mL of the reagent solution (H_2SO_4, sodium phosphate and ammonium molybdate). In

case of blank, 0.3mL of msethanol was used in place of sample. The tubes were incubated for 95°C for 90min. After the mixture was cooled to room temperature, the absorbance was measured at 695nm against blank. Ascorbic acid was used as a standard and was treated in a similar manner. The total antioxidant activity is expressed as the number of equivalents of ascorbic acid (µg/g). The results were tabulated in **table 15** and the graphical representation is presented in **Fig. 22.**

Method 3: Reducing power assay [134]

Principle:

This is a spectrophotometric method and is based on the principle that an increase in absorbance of the reaction mixture as concentration increase indicates an increased antioxidant activity.

The assay is based on the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the formation of Prussian blue colour complex when treated with ferric chloride. The absorbance of the blue complex is measured at 700nm.

Instrument

Shimadzu UV Visible spectrophotometer. Model 1800

Reagents

1% potassium ferricyanide

10% trichloro acetic acid.

0.2M, phosphate buffer (pH 6.6)

0.1% ferric chloride.

Procedure

Various concentration of methanolic extracts of *Citrullus lanatus* was mixed with 0.75mL phosphate buffer and 0.75mL potassium ferricyanide [K_3 Fe(CN_6)], then the mixture

was incubated at 50°C for 20 min. 0.75mL of trichloro acetic acid was added to the mixture, which was then centrifuged at 3000rpm for 10min. Finally 1.5mL of the supernatant solution was mixed with 1.5mL of distilled water and 0.1mL of ferric chloride ($FeCl_3$) and absorbance was measured at 700nm in a UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used as blank. The absorbance of the final reaction mixture of three parallel experiments was expressed as mean ± standard error of mean. Increased absorbance of the reaction mixture indicates stronger reducing power. The results obtained are tabulated in **table 16** and the graphical representation is presented in **Fig. 23.**

Method 4: Ferric Reducing Antioxidant Power (FRAP) Assay [135]

Total antioxidant activity is measured by FRAP assay of Benzie *et al.,* 1999. The ferric reducing antioxidant power assay measures the potential of antioxidants to reduce the Fe^{3+} and 2,4,6 tripyridyl-s-triazyne (TPTZ) complex present in stoichiometric excess to the blue coloured Fe^{2+} complex which increases the absorbance at 593nm.

Principle

At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) can be monitored by measuring the change in absorption at 593nm. The reaction is non-specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe III to Fe II) ion formation. The change in absorbance is therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture.

Instrument

Shimadzu UV Visible spectrophotometer, Model 1800

Reagents

FRAP Reagent

a) Acetate buffer 30mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water.

b) TPTZ (2, 4, 6-tripyridyl-s- triazine) (M.W. 312.34) 10mM in 40mM HCl

c) $FeCl_3$. $6H_2O$ (M.W. 270.30) 20mM

The working FRAP reagent was prepared freshly by mixing a, b & c in the ratio of 10:1:1 at the time of use.

Procedure

The methanolic extract of *Citrullus lanatus* was dissolved in methanol to get a stock solution containing 1mg/mL. Varying quantities of the stock solution were added to 3mL of working FRAP reagent and absorbance was measured at 0min after vortexing at 593nm. Thereafter samples were placed at 37ºC in water bath and absorption was again measured after 4min. Ascorbic acid was used as standard. The result obtained for the FRAP assay are presented in the **table 17** and **Fig.24.**

SECTION B - LARVICIDAL ACTIVITY OF METHANOLIC EXTRACT OF *CITRULLUS LANATUS*[136-147]

Malaria and other vector-borne diseases contribute to the major disease burden in India. One of the methods to control these diseases is to control the vectors for the interruption of disease transmission. In the past, synthetic organic chemical insecticides based intervention measures for the control of insect pests and disease vectors have resulted in development of insecticide resistance in some medically important vectors of malaria, filariasis, Japanese encephalitis, dengue, hemorrhagic fever, chikungunya and yellow fever transmitted by mosquitoes which cause millions of death every year. Hence destroying mosquitoes is one method for preventing the above infections [147-148]. During the last decade,

various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides.

The present study has attempted to study mosquito larvicidal property of *C. lanatus* against three mosquito species - *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae).

Preparation of stock solution of methanolic extract

A stock solution of 100mg/mL of the methanolic extract of the plant *Citrullus lanatus* was prepared by dissolving the required quantity of the extract in distilled water.

Procedure for extract

The method adopted by Arivoli S and Samuel Tennyson 2012 was followed. The larvicidal activity of plant extract was carried out on late 3rd and early 4th instar larvae of ***Anopheles stephensi,*** a primary vector of urban malaria, ***Culex quinquefaciatus***, a common vector of filariasis, ***Aedes aegypti***, common vector of dengue and yellow fever. The mosquito larvae were obtained from ICMR, Madurai. Twenty larvae were released in 500mL beaker containing 200mL distilled water with varying concentrations of plant extract. The larvae were provided with dog biscuit and yeast powder in a ratio of 3:2 as nutrients. The experiments were carried out a room temperature (26°C ± 2°C). Three replicates of each concentration were run under the same microclimate conditions along with untreated control. The mortality was monitored for 24h. The results obtained are presented in **Table 18 to 20** and **Fig. 25 to 27.**[148].

Data analysis[149-150]

LC50 (lethal concentration to cause 50% mortality in the population) and LC90 (lethal concentration to cause 90% mortality in the population) were determined by plotting the regression line as described by Finney. The percentage mortality was calculated using Abbot's formula and the data so obtained was analyzed by probit analysis (Finney 1989) by

using the software Minitab-15 for dose and time mortality regression lines. The purpose of the probit transformation is to straighten the line so we can estimate LC 50 more easily.

$$\text{Percentage Mortality} = \frac{\%\text{Mortality in treated} - \%\text{Mortality in control}}{100 - \%\text{ Mortality in control}} \times 100$$

SECTION C - PANCREATIC LIPASE INHIBITION ASSAY[151-153]

Obesity is a medical complication caused by an imbalance between energy intake and expenditure and is broadly recognized as a major public health problem. Obesity can lead to variety serious diseases, including hypertension, hyperlipidemia, atherosclerosis, and type II diabetes and thus indirectly leading to aging. The inhibitors of digestive lipases reduce dietary fat absorption and hence act as anti-obesity agents. New Pancreatic lipase inhibitors derived from natural sources especially from medicinal herbs are used for the treatment of obesity.

Pancreatic lipase

Pancreatic lipase (PL) is an enzyme, secreted from the pancreas and plays an excellent role in the absorption of triglyceride in the small intestine. Dietary fats are composed of about 95% triacylglycerols (TG). Pancreatic lipase hydrolyses the water insoluble triacylglycerols in the intestinal lumen and thereby used for the dietary fat absorption.

Pancreatic lipase inhibitors

Pancreatic lipase inhibitors are considered to be a valuable therapeutic agent for treating diet-induced obesity in humans as anti-obesity agents. Grape seed extracts, carnosic acid from the ethanolic extract of leaves of *Salvia officinalis* L (sage), flavan dimmers isolated from fruits of *Cassia nomame* (Leguminosae), the methanol extract of *Dioscorea* nipponica,5-hydroxy-7-(41-hrdroxy-31-methoxyphenyl)-l-phenyl-3-heptone (HPH) and 3-methylethergalangin from the rhizome of *Alpinia officinarum* are examples of pancreatic lipase inhibitors of plant sources

Principle Lipases have certain roles in human pathogenesis and are a key enzyme catalyzes the hydrolysis of emulsified esters of glycerol and long chain fatty acids. Short chain fatty acids can be directly absorbed into the blood, while long-chain fatty acids and monoglycerides combine with bile salts to form water soluble micelles. The micelles are absorbed into the mucosal cells of the intestine and the fatty acids and monoglycerides are resynthesized into triglycerides. Dietary triglyceride is usually stored in the adipose tissue. Pharmacological agents that reduce the absorption of dietary triglycerides, reduce the probability of the formation of atherosclerotic plaque. Triglyceride hydrolysis by pancreatic lipase is inhibited by physiological concentrations of bile salts.

An anti-obesity drug Orlistat, inhibits pancreatic lipase in the lumen of the gastrointestinal tract to decrease systemic absorption of dietary fat.

Requirements

- Chicken (*Gallus domesticus*) pancreas
- Sucrose solution (0.01M)
- Ammonium sulphate (50% saturation)
- Phosphate buffer (pH7)
- Olive oil
- Pancreatic lipase
- Orlistat (60mg)

Procedure [153]

Extraction of Lipase from Chicken (*Gallus domesticus*) pancreas

Pancreas of freshly slaughtered chicken was collected with the guidance of a veterinary surgeon. It was washed thoroughly and pancreatic lipase was placed in ice cold

sucrose solution (0.01M). The Pancreas was homogenized in 0.01M sucrose and centrifuged. The supernatant solution was separated and subjected to ammonium sulphate precipitation (50% saturation). The obtained white pellets after centrifugation was dissolved in sucrose solution and again saturated with 50% ammonium sulphate and centrifuged. Finally pellets were used as enzyme source by dissolving in phosphate buffer (pH 7).

Determination of chicken pancreatic lipase activity

The chicken pancreatic lipase activity was determined by incubating an emulsion containing 8mL of olive oil (Dietary fat), 0.4mL of phosphate buffer and 1 mL of chicken pancreatic lipase for an hour. The reaction was stopped by addition of 1.5mL of a mixture containing acetone and 95% ethanol (1:1). The amount of liberated fatty acid was determined by titrating the emulsion against 0.02M NaOH (standardized by potassium hydrogen phthalate) using phenolphthalein as an indicator. The end point is the appearance of pink colour. The volume of sodium hydroxide consumed was taken as (A).

Pancreatic lipase inhibitory activity

Pancreatic lipase inhibitory activity has been studied using Prashith Kekuda T R et al. method with slight modification. The plant extract of *Citrullus lanatus* was prepared in different concentrations such as 1.0mg/mL, 2.0mg/mL, 3.0mg/mL, 4.0mg/mL, 5.0mg/mL. A 100µL of each concentration of sample was mixed with 8 mL of olive oil, 0.4 mL phosphate buffer and 1 mL of Chicken pancreatic lipase and it was incubated for 60mins. The reaction was stopped by the addition of 1.5 mL of a mixture containing acetone and 95% ethanol (1:1). Appearance of pink colour from yellow colour shows the liberated fatty acids, which was determined by titrating the solution against 0.02M NaOH (standardized by potassium hydrogen phthalate) using phenolphthalein as an indicator and the percentage inhibition of lipase activity was calculated using the following formula , **Lipase inhibition = [A-B/ B]** ×

100, where A - Lipase activity, B - Activity of lipase when incubated with the standard and test compounds and the results are tabulated in **Table 21** and the pictorial representations are presented in **Fig.28.**

SECTION D - *IN VITRO* ANTI CANCER ACTIVITY (BREAST CANCER ACTIVITY BY USING HUMAN BREAST CANCER CELL LINES) [154-156]

Cancer is a group of many different diseases that have some important things in common. They all arise in cells, the body's unit of life. The body is made up of many types of cells. Normally, cells grow and divide to produce more cells only when the body needs them. Sometimes cells keep dividing when new cells are not needed. These cells may form a mass of extra tissue called a growth or tumor. Tumors can be benign or malignant.

Benign tumors are not cancer. They can usually be removed, and in most cases, they don't come back. Most important, the cells in benign tumors do not invade other tissues and do not spread to other parts of the body. Benign tumors are not a threat to life.

Malignant tumors are cancer. Cells in these tumors can invade and damage nearby tissues and organs. Also cancer cells can break away from a malignant tumor and enter the bloodstream or lymphatic system. That is how breast cancer spreads and forms secondary tumors in other parts of the body. The spread of cancer is called metastasis.

Breast cancer

The most common type of breast cancer begins in the lining of the ducts and is called ductal *carcinoma*. Another type, called lobular carcinoma, arises in the lobules.The risk of breast cancer increases gradually as a woman gets older. The risk factors for breast cancer include age, personal and family health history, genetic changes, prior radiation therapy, reproductive and menstrual history, race, breast density, overweight and obesity, physical inactivity, and alcohol consumption. The various symptoms include a lump or thickening in

or near the breast or underarm area, change in the size or shape of the breast, fluid discharge from the nipple, especially if it's bloody

Treatment

Surgery is the most common treatment for breast cancer. An operation to remove the breast (or as much of the breast as possible) is a *mastectomy*. An operation to remove the cancer but not the breast is called breast-sparing surgery or breast-conserving surgery. *Lumpectomy* and *segmental mastectomy* (also called partial mastectomy) are types of breast-sparing surgery. They usually are followed by radiation therapy to destroy any cancer cells that may remain in the area. In most cases, the surgeon also removes lymph nodes under the arm to help determine whether cancer cells have entered the *lymphatic system*.

Radiation therapy is the use of high-energy rays to kill cancer cells and stop them from growing. The therapy may be by external source or implant radiation. Some women receive both kinds of radiation therapy.

Chemotherapy is the use of drugs to kill cancer cells. It is generally a combination of drugs. The drugs may be given by mouth or by injection. It is a systemic therapy because the drugs enter the bloodstream and travel throughout the body.

Hormonal therapy is used to keep cancer cells from getting the hormones they need to grow. This treatment may include the use of drugs that change the way hormones work or surgery to remove the ovaries, which make female hormones. It is also a systemic treatmentand hence can affect cancer cells throughout the body.

Advantages of Herbal Medicines compare with alternative therapy [157]

Herbal medicine is one of the most commonly used complementary and alternative therapies (CAM) by people with cancer. Some studies have shown that as many as 6 out of every 10 people with cancer (60%) use herbal remedies alongside conventional cancer treatments.

Cell line

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetraacetic acid (EDTA) to make single cell suspension and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay[158]

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48h of incubation, 15µL of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows - % Cell viability = [A] Test / [A]control x 100. The results are tabulated in **Table 22 & 23** and Photomicrograph of human breast cancer cell lines (MCF-7) presented in **Fig. 29 & 30.**

SECTION E - *IN VITRO* ANTI DIABETIC ACTIVITY [159-163]

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydispsia (increased thirst) and polyphagia (increased hunger). Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha Glucosidase.

Three main types of Diabetes mellitus (DM)

- Type 1 DM results from the body's failure to produce insulin, and currently requires the person to inject insulin or wear an insulin pump. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes".
- Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was previously referred to as non insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes".
- The third main form, gestational diabetes, occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may precede development of type 2 DM.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

Symptoms

The symptons include high levels of sugar in the blood, unusual thirst, frequent urination, extreme hunger and loss of weight, blurred vision, nausea and vomiting, extreme weakness and tiredness, irritability, mood changes etc.

Complications

The ccomplications of diabetes include eye, foot, skin complications, heart problems, hypertension, mental health, hearing loss, gum disease, gastroparesis, ketoacidosis, neuropathy, nephropathy, peripheral arterial disease, stroke, erectile dysfunction, infections and non healing of wounds

Medicinal plants used in treatment of diabetes

These include *Allium sativum*, *Eugenia jambolana*, *Momordica charantia Ocimum sanctum*, *Phyllanthus amarus*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Trigonella foenum graecum* and *Withania somnifera*.

Herbal formulations in the treatment of diabetes mellitus [164]

Diabetes mellitus in Ayurveda is known as Madhu-meha. Several Ayurvedic formulations have been used in the treatment of Diabetes mellitus for centuries. In addition to herbs, minerals find wide application in Ayurvedic prescription for diabetes. Medicinal herbs like *Momordica charantia, Gymnema sylvestre, Enicostermma littorale, Pterocarpus marsupium, Salacia reticulate, Coccinia gluaca* and *Trigonella foneum graceum* are prescribed as single powder drugs or in combination (poly-herbal).

Rasayana is an important branch of Ayurveda. The main goal of Rasayana therapy is better quality of life with increased lifespan. Rasayana includes drug formulation, dietary regimen and code of conduct. Many of the drugs used in Rasayana therapy in diabetes mellitus have excellent antioxidant properties, like *Phyllanthus emblica, Azadirachta indica, Ocium sanctum and Tinospora cordifolia*. The Rasayana approach to treat diabetes consists of **Aeara Rasayana** (antistress), **Ajasrika Rasayana** (dietary control), **Osad Rasayana** (Preventive), **Naimittika Rasayana** (hypoglycemic).

Significance of herbal drugs compare with Allopathic Drugs

Medicinal plants are being looked up once again for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. Metformin exemplifies an efficacious oral glucose-lowering agent. Its development was based on the use of *Galega officinalis* to treat diabetes. *Galega officinalis* is rich in guanidine, the hypoglycemic component. Because guanidine is too toxic for clinical use, the alkyl

biguanides synthalin A and synthalin B were introduced as oral anti-diabetic agents in Europe in the 1920s but were discontinued after insulin became more widely available. However, experience with guanidine and biguanides prompted the development of metformin.

To date, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated.

Advantages of herbal medicine over Allopathic drugs

In India, it is proving to be a major health problem, especially in the urban areas. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects, low cost, widely available and less toxic compared with allopathic drugs.

The present study of *in-vitro* anti-diabetic activity was carried out by following methods

- Non-enzymatic glycosylation of haemoglobin Assay.
- Glucose uptake in yeast cells
    - % inhibition of Glucose uptake in 5mM glucose concentrations.
    - % inhibition of Glucose uptake in 10mM glucose concentrations.
- Alpha amylase inhibition assay.
- Alpha glucosidase inhibition assay

METHOD I: NON-ENZYMATIC GLYCOSYLATION OF HAEMOGLOBIN ASSAY

Glucose reacts non enzymatically with the NH2-terminal amino acid of the beta chain of human hemoglobin by way of a ketoamine linkage, resulting in the formation of hemoglobin AIc. Other minor components appear to be adducts of glucose 6-phosphate and fructose 1,6-diphosphate. These hemoglobins are formed slowly and continuously throughout the 120-day life-span of the red cell. There is a two- to threefold increase in hemoglobin AIc in the red cells of patients with diabetes mellitus. By providing an integrated measurement of blood glucose, hemoglobin AIc is useful in assessing the degree of diabetic control. Furthermore, this hemoglobin is a useful model of non-enzymatic glycosylation of other proteins that may be involved in the long-term complications of the disease.[165]

The incubation of dialyzed hemoglobin with a number of phosphorylated glycolytic intermediates leads to the formation of covalent hemoglobin adducts that co-chromatograph with haemoglobin. From 7 to 12% of the hemoglobin can be modified after a 72-h incubation of an equimolar mixture of haemoglobin and the phosphorylated intermediate. The concentration of haemoglobin is elevated in patients with Diabetes mellitus. This presumably reflects the increased concentrations of glycolytic intermediates, which were found to be significantly elevated in the red cells of diabetic patients as compared with normal controls.[166]

Principle

Human bodies possess enzymatic and non- enzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes. Increased concentration of glucose in the blood leads to its binding to hemoglobin which may result in the formation of the reactive oxygen species. An

increase in the glycosylation was observed on incubation of hemoglobin with the increasing concentration of the glucose over a period of 72h. [167-168]

Reagents

- 2% glucose solution.
- 0.06% haemoglobin solution.
- 0.02% gentamycin solutions.
- 0.01 M phosphate buffer (pH 7.4)

Procedure

Anti diabetic activity of methanolic extract of the leaves of *Citrullus lanatus* were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured spectrophotometrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in 0.01M phosphate buffer (pH 7.4). 1mL each of above solution was mixed with 1mL of various concentrations of the extract. The mixture was incubated in dark at room temperature for 72h. The degree of glycosylation of haemoglobin was measured spectrophotometrically at 520nm. α-tocopherol (Trolax) was used as a standard drug for assay. The percentage inhibition was calculated. All the tests were performed in triplicate. [169-170]

The % inhibition was calculated using the following formula,

$$\% \text{ inhibition} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where **Abs control** is the absorbance of the control and **Abs sample** is the absorbance of the test sample. The results are tabulated in **Table 24** and **Fig. 33.**

METHOD II*: IN VITRO* GLUCOSE UPTAKE IN YEAST CELLS

The rate of glucose transport across cell membrane in yeast cells system is presented. Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species. In Yeast glucose transport takes place through facilitated diffusion. After the treatment of the yeast cells with extract, the glucose uptake was found to increase in a dose dependent manner. The methanolic extract of *Citrullus lanatus* glucose uptake by yeast cells as compared to standard drug acarbose. [171-172]

Principle

In the glucose uptake in yeast cells method the glucose transport across the yeast cell membrane has been receiving attention as in vitro screening method for hypoglycaemic effect of various compounds/ medicinal plants. The transport of non metabolizable sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers. It is reported that in yeast cells glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast is by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose.

Reagents [171]

- Baker's yeast.
- Glucose solution (5 and 10mM)
- GOD solution

Procedure

Yeast cells were prepared by commercial baker's yeast was washed repeated centrifugation (3,000 rpm; 5min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. 1mL of various concentrations of methanolic extract of *Citrullus lanatus* were added to 1mL of glucose solution (5 and 10mM) and incubated together for 10min at 37°C. Reaction was started by adding 100µL of yeast suspension, vortexed and further incubated at 37°C for 60min. After 60min, the tubes were centrifuged (2,500 rpm, 5min) and glucose was estimated in the supernatant by glucose oxidase method. The optical density 520 nm was measured. Acarbose was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula,

$$\textbf{Increase in glucose uptake (\%)} = \frac{\textbf{Abs sample} - \textbf{Abs control}}{\textbf{Abs sample}} \textbf{X 100}$$

Where, **Abs control** is the absorbance of the control and **Abs sample** is the absorbance of the test sample. All the experiments were carried out in triplicates. The results are tabulated in **Table 25,26** and **Figs. 34, 35.**

METHOD III: *IN VITRO* ALPHA AMYLASE INHIBITION ASSAY

The α-amylase is the one of the main enzymes in human that catalyses the hydrolysis of 1,4-glucosidic linkage of complex carbohydrates like starch into simple sugars namely, maltose. Inhibition of the α-amylase activity is one of the possible six mechanisms that can be potentially used for controlling diabetes.

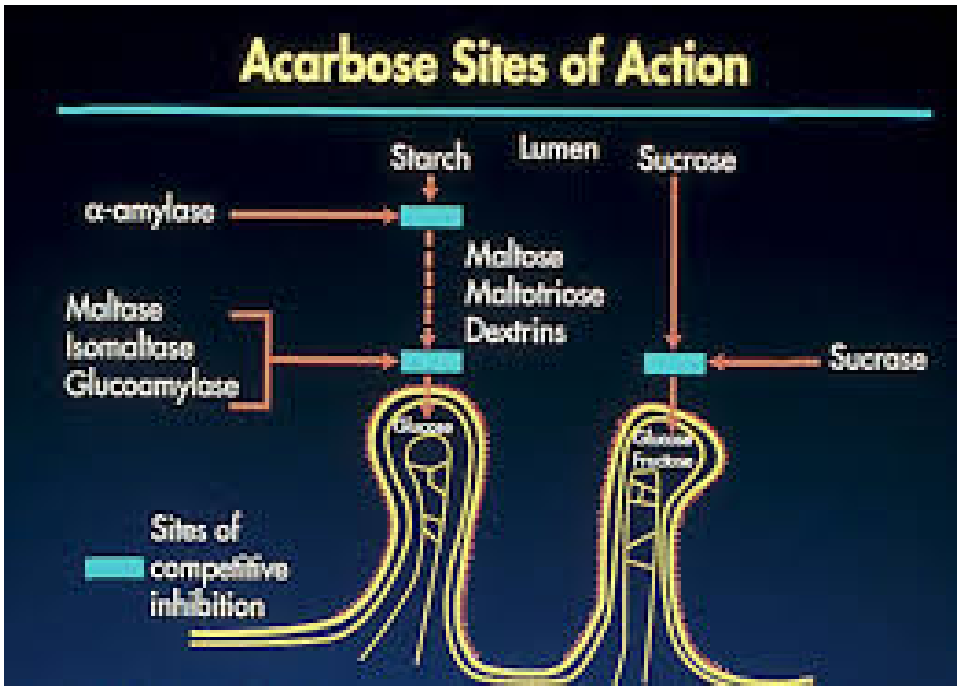
Controlling the glucose production from complex carbohydrates is considered to be effective in controlling diabetes. Although the inhibitory drug like acarbose is which inhibits α-amylase and α-glucosidase used in controlling glucose level in type 2 diabetic patients, the

acarbose has undesirable side effects, especially flatulence and diarrhea. Natural products extracted from plants are known to controlling hyperglycemia. The monoterpenes, sesquiterpenes, and oxygenic derivatives in herbal plant extracts are known to inhibit the activity of carbohydrate hydrolyzing enzymes. [173]

Principle

Alpha amylase is the important enzymes involved in the digestion of carbohydrates. Alpha Amylase is involved in the breakdown of long chain carbohydrates. It serves as the major digestive enzymes and help in intestinal absorption. α-amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugars. This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood. Hence diabetics tend to have low alpha amylase levels in order to keep their glucose levels under control. Therefore alpha amylase inhibitors have potential roles in controlling blood sugar levels and crop protection. [174-177].

Fig.31: Acarbose sites of action



Reagents

- 0.02M sodium phosphate buffer (pH 6.9 containing 6mM sodium chloride)
- α-amylase solution (27.5mg of α-amylase in 100ml 0f water)
- 1%, w/v soluble starch
- 1M HCl
- Iodine reagent (5mM I_2 and 5mM KI)

Procedure

Various concentrations of methanolic extract of *Citrullus lanatus* 500µL were added to 500µL of 0.02M sodium phosphate buffer (pH 6.9 containing 6mM sodium chloride) containing 500µL of α-amylase solution and were incubated at 37°C for 10min. Then 500µL soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15min. 1M HCl (20µL) was added to stop the enzymatic reaction, followed by the addition of 100µL of iodine reagent. The colour change was noted and the absorbance was read at 620nm. Acarbose was used as reference. Inhibition of enzyme activity was calculated as: **Inhibition of enzyme activity (%) = (C-S) / C × 100,** where S is the absorbance of the sample and C is the absorbance of blank (no extract).[190] **.** The results are tabulated in **Table 27** and **Fig. 36.**

METHOD IV: *IN VITRO* ALPHA GLUCOSIDASE INHIBITION ASSAY

α-glucosidase catalyzes the final step in the digestive process of carbohydrates. Its inhibitors can retard the uptake of dietary carbohydrates and suppress postprandial hyperglycemia and could be useful for treating diabetic and/or obese patients (Toeller, 1994). α-Glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce postprandial hyperglycemia primarily by interfering with the carbohydrate digestive enzymes and by delaying glucose absorption.

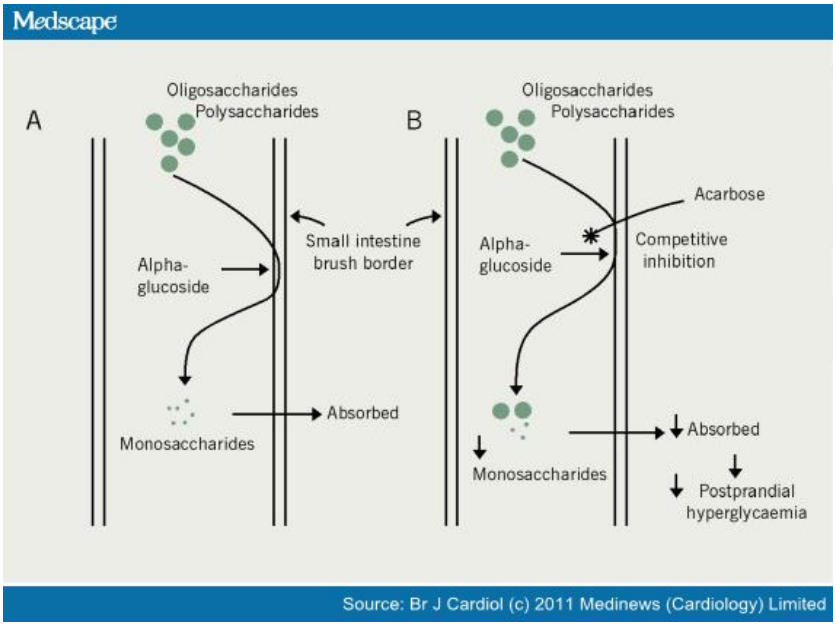
Acarbose a carbohydrate inhibitor, when administered showed delayed in glucose absorption Acarbose, an α-Glucosidase inhibitors, reduce intestinal absorption of

carbohydrate and there by blunt the postprandial rise in plasma glucose in diabetic patients. However flatulence and abdominal due to the malabsorption limits its potential as favoured medication.

Principle

α-Glucosidase inhibitors are among the available glucose lowering medications. This enzyme is located in the brush border of the small intestine and is required for the breakdown of carbohydrates to absorbable monosaccharide. The α-Glucosidase inhibitors delay but do not prevent the absorption of ingested carbohydrates but reduce the postprandial glucose and insulin peak. [178-181].

Fig. 32: Mechanism of action of Alpha-glucosidase



Reagents

- α- glucosidase (1U/ml).
- 0.2M Tris buffer (pH 8.0).
- GOD solution.

Procedure

The enzyme α- glucosidase inhibitory activity is determined by incubating solution (0.1 ml) of an enzyme preparation with 0.2 M Tris buffer, pH 8.0 (1.0ml) containing 1mL of various concentrations of extract at 37 ºC for 60 minutes. The reaction mixture is heated for two minutes in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidation method. Acarbose was used as reference. [182-183]. The results are tabulated in **Table 28** and **Fig. 37.**

$$\% \text{ inhibition} = \frac{\text{(Enzyme activity of control – Enzyme activity of extract)}}{\text{Enzyme activity of control}} \times 100$$

RESULTS AND DISCUSSION

SECTION A – IN VITRO ANTIOXIDANT ACTIVITY

The in-vitro antioxidant activity of the plants was studied by four methods. The results obtained for these methods are presented in **Tables** and the graphical representations are presented in **Figs.**

Method I: Free radical Scavenging activity using 2,2-Diphenyl-1-Picryl hydrazyl (DPPH) free radical.

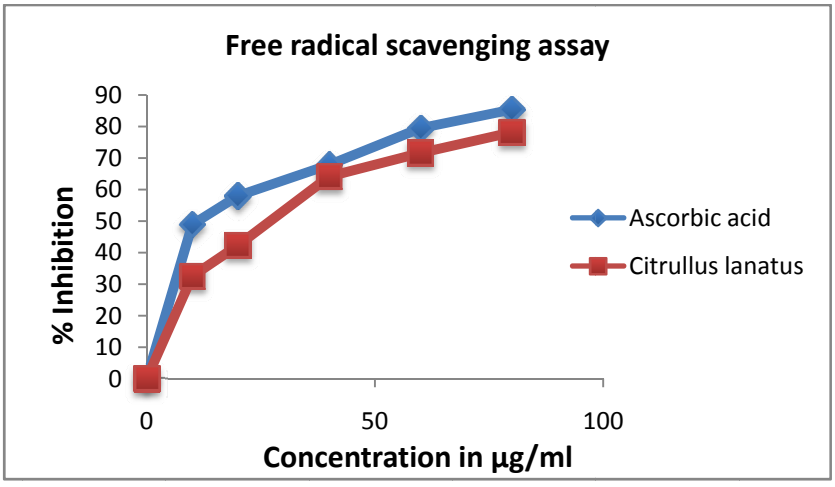
The results obtained for the free radical scavenging activity against DPPH radical is presented in **Table 14** and the graphical representation is presented in **Fig.21.**

Table 14: Percentage inhibition of methanolic extract of *C. lanatus* and standard ascorbic acid against DPPH at 517nm

S. No.	Conc. in µg/mL	Percentage inhibition by ascorbic acid	Percentage inhibition by *Citrullus lanatus*
1	10	48.91 ± 0.60	32.55 ± 0.32
2	20	58.03 ± 0.50	42.32 ± 0.48
3	40	67.86 ± 0.27	64.14 ± 0.61
4	60	79.49 ± 0.30	71.63 ± 0.29
5	80	85.36 ± 0.29	78.16 ± 0.29
	IC_{50}	**27.29 µg/ml**	**37.12 µg/ml**

***mean of three readings ± SEM**

Fig.21: Free radical scavenging assay of ascorbic acid and methanolic extract of *C. lanatus* against DPPH at 517nm



From the **table 14,** it can been seen that the methanolic extract of *C. lanatus* showed a percentage inhibition of 78.16 ± 0.29 while ascorbic acid showed a percentage inhibition of 85.36 ± 0.29 at a concentration of 80µg/mL. The IC_{50} value calculated using the linear regression analysis was found to be 37.12 and 27.29µg/mL for methanolic extract and ascorbic acid respectively. The extract possessed a good radical scavenging capacity

Method 2: Antioxidant activity by Phosphomolybdenum method

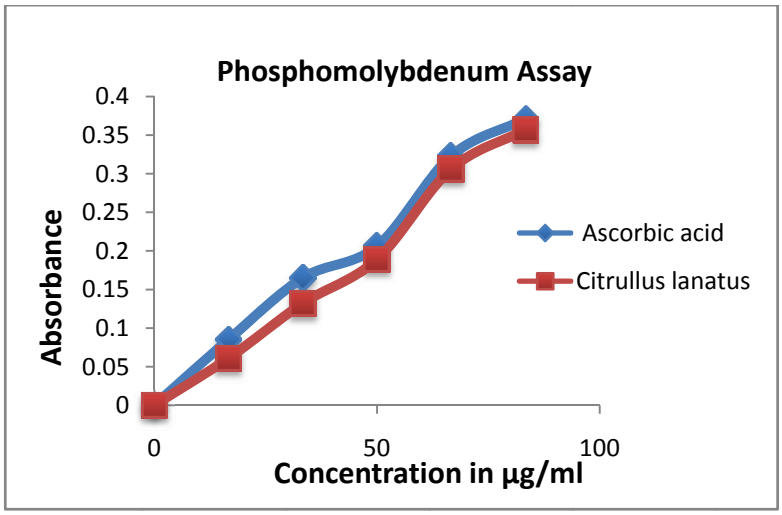
The results obtained for the phosphomolybdenum activity of methanolic extract of C.lanatus and standard ascorbic acid are tabulated in **table 15** and the graphical representation is presented in **Fig.22.**

Table 15: Absorbance of methanolic extract of *C. lanatus* and standard ascorbic acid in Phosphomolybdenum method

S. No.	Conc. in µg/mL	Absorbance of Ascorbic acid	Absorbance of *Citrullus lanatus*
1	16.66	0.085 ± 0.005	0.061 ± 0.002
2	33.33	0.165 ± 0.004	0.132 ± 0.004
3	50.00	0.206 ± 0.008	0.189 ± 0.009
4	66.66	0.323 ± 0.004	0.306 ± 0.004
5	83.33	0.371 ± 0.005	0.357 ± 0.005

***Mean of three readings ± SEM**

Fig.22 : Antioxidant activity by Phosphomolybdenum method



From the **table 15** and **Fig.22,** it can be seen that the extract possessed a reducing capacity similar to the ascorbic acid and both of them showed an increase in absorbance.

Method 3: Reducing Power Assay

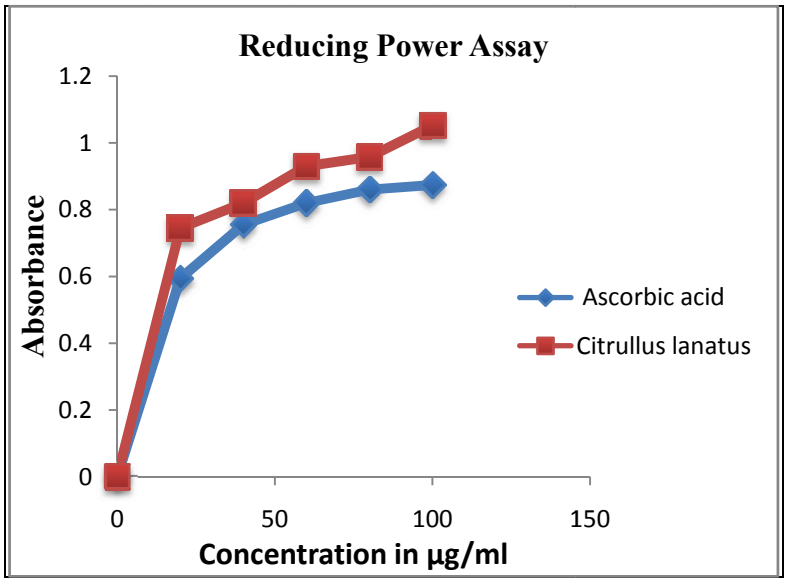
The results obtained for the Reducing Power assay of methanolic extract of C.lanatus and standard ascorbic acid are presented in **table 16** and the graphical representation is presented in **Fig.23.**

Table 16: Absorbance of methanolic extract of *C. lanatus* and standard ascorbic acid in Reducing power aaasy

S. No.	Conc. in µg/mL	Absorbance of Ascorbic acid	Absorbance of *C. lanatus*
1	20	0.745 ± 0.012	0.594 ± 0.005
2	40	0.820 ± 0.003	0.755 ± 0.006
3	60	0.930 ± 0.002	0.820 ± 0.003
4	80	0.958 ± 0.059	0.860 ± 0.003
5	100	1.052 ± 0.007	0.874 ± 0.007

***Mean of three readings ± SEM**

Fig.23 : Antioxidant activity by Reducing Power Assay



Method 4: Ferric reducing antioxidant power assay (FRAP assay)

The results obtained for the Ferric Reducing Antioxidant Power assay are presented in **Table.17.**

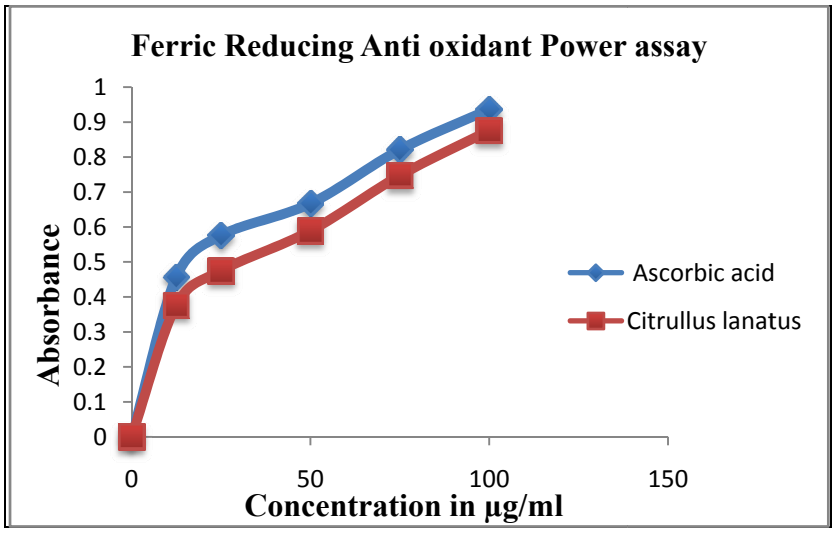
Table 17: Ferric reducing anti-oxidant power assay of ascorbic acid and methanolic extract of *C.lanatus*

S. No.	Conc. in µg/mL	Absorbance of Ascorbic acid	Absorbance of *C. lanatus*
1	12.5	0.457 ± 0.001	0.376 ± 0.002
2	25	0.576 ± 0.004	0.475 ± 0.005
3	50	0.667 ± 0.003	0.589 ± 0.003
4	75	0.821 ± 0.001	0.747 ± 0.002
5	100	0.936 ± 0.002	0.876 ± 0.003

***Mean of three readings ± SEM**

From the **table 17,** it can be seen that the methanolic extract of *C. lanatus* showed an absorbance of 0.876 ± 0.003 for a concentration of 100µg/mL while ascorbic acid showed an absorbance of 0.936 ± 0.002 at a concentration of 100µg/mL. The extract showed a dose dependent reducing ability. The graphical representations of the reducing power activity of the methanolic extract of *C. lanatus* and ascorbic acid are presented in **Fig.24.**

Fig. 24: Ferric reducing anti-oxidant assay ofmethanolic extract of *C. lanatus*



The plant extract possess good antioxidant activity and compared with the standard ascorbic acid.

SECTION B - LARVICIDAL ACTIVITY OF METHANOLIC EXTRACT *OF CITRULLUS LANATUS*

The results obtained for the larvicidal effect of methanolic extract of *Citrullus lanatus* are presented in **Tables 18 to 20** and the graphical representations are presented in **Figs. 25 to 27.**

Table 18: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Anopheles stephensi*

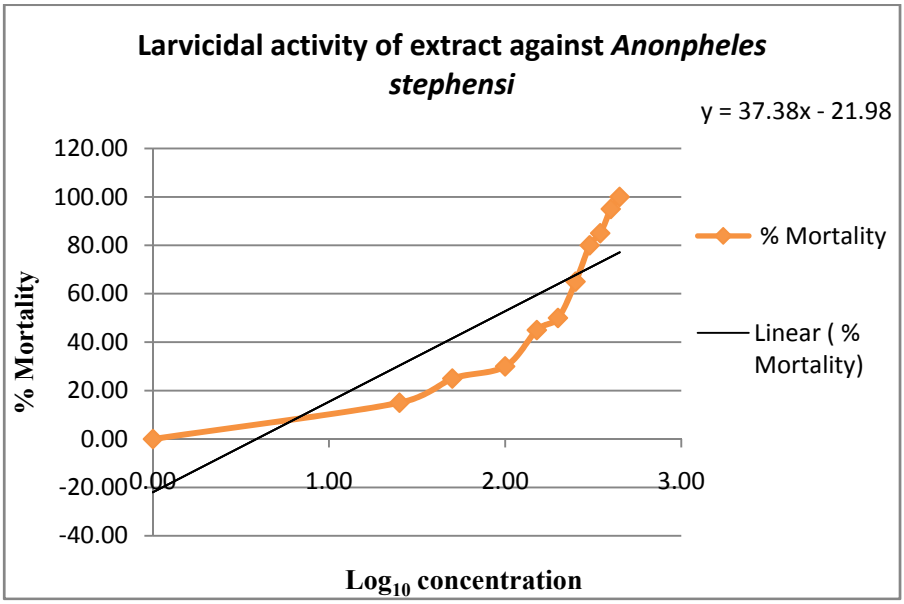
S. No	Conc. (ppm)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Probit	Probit	Regression value
0	0	0	0	0	0	0		0
1	25	1.40	20	3.00	15.00	3.77	3.00	4.358
2	50	1.70	20	5.00	25.00	4.19	4.00	4.778
3	100	2.00	20	6.00	30.00	4.36	4.00	4.948
4	150	2.18	20	9.00	45.00	4.8	5.00	5.388
5	200	2.30	20	10.00	50.00	4.92	5.00	5.508
6	250	2.40	20	13.00	65.00	5.33	6.00	5.918
7	300	2.48	20	16.00	80.00	5.81	6.00	6.398
8	350	2.54	20	17.00	85.00	5.99	6.00	6.578
9	400	2.60	20	19.00	95.00	6.65	7.00	7.238
10	450	2.65	20	20.00	100.00			
LC50				**84.23ppm or 0.084v/v**				
LC90				**989.396ppm or 0.989%v/v**				

The LC50 (lethal concentration to cause 50% mortality in the population) and LC90 (lethal concentration to cause 90% mortality in the population) were determined by plotting the regression line as described by Finney . The percentage mortality was calculated using Abbot's formula and the data so obtained was analyzed by probit analysis (Finney 1989) by using the software Minitab-15 for dose and time mortality regression lines. The purpose of the probit transformation is to straighten the line so we can estimate LC 50 more easily.

From the **Table 18** it can be observed that a mortality of 100.00 ± 0.00 was observed for *Anopheles stephensi.* The LC_{50} and LC_{90} values were calculated using Probit analysis.

The percentage mortality calculated using Abbott's formula versus log concentration was plotted and Y=50 is substituted in the resulting linear equation to obtain the X value. The linear regression equation was found to be y = 37.387x - 21.988 for activity against *Anopheles stephensi.* The antilog of X was then the LC_{50} (conc. of 50% mortality) or LC_{90} (conc. of 90% mortality) value.

Fig. 25: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Anonpheles stephensi*



The LC_{50} was found to be **84.23ppm or 0.084v/v** for *Anopheles stephensi*. The LC_{90} value was found to be **989.3956ppm or 0.989%v/v** for *Anopheles stephensi*. One hundred percent mortality was observed for the concentrations tested against the organisms. The extract was effective against *Anopheles stephensi.*

From t**able 19,** it can be observed that a mortality of 100.00 ± 0.00 was observed for *Culex quinquefasciatus.* The LC_{50} and LC_{90} values were calculated using Probit analysis. The percentage mortality calculated using Abbott's formula versus log concentration was plotted and Y=50 is substituted in the resulting linear equation to obtain the X value.

Table 19: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Culex quinquefasciatus*

S. No	Conc. (ppm)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Probit	Probit	Regression value
0	0	0	0	0	0	0	0	0.00
1	5	0.70	20	1.00	5.00	0	0.00	0.65
2	10	1.00	20	1.00	5.00	0	0.00	0.65
3	25	1.40	20	3.00	15.00	3.77	4.00	4.42
4	50	1.70	20	6.00	30.00	4.36	4.00	5.01
5	100	2.00	20	10.00	50.00	4.92	5.00	5.57
6	150	2.18	20	13.00	65.00	5.33	5.00	5.98
7	200	2.30	20	16.00	80.00	5.81	6.00	6.46
8	250	2.40	20	17.00	85.00	5.99	6.00	6.64
9	300	2.48	20	19.00	95.00	6.75	7.00	7.40
10	350	2.54	20	20.00	100.00			
LC50				**51.31ppm or 0.051%v/v**				
LC90				**405.88ppm or 0.405%v/v**				

The linear regression equation was found to be y = 44.533x - 26.161 for activity against *Culex quinquefasciatus.* The antilog of X was then the LC_{50} (conc. of 50 %mortality) or LC_{90} (conc. of 90% mortality) value. The LC_{50} was found to be **51.31ppm or 0.051%v/v** for *Culex quinquefasciatus*. The LC_{90} value was found to be **405.88ppm or 0.405%v/v** for *Culex quinquefasciatus*. One hundred percent mortality was observed for the concentrations tested against the organisms. The extract was effective against *Culex quinquefasciatus*

Fig. 26: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Culex quinquefasciatus*

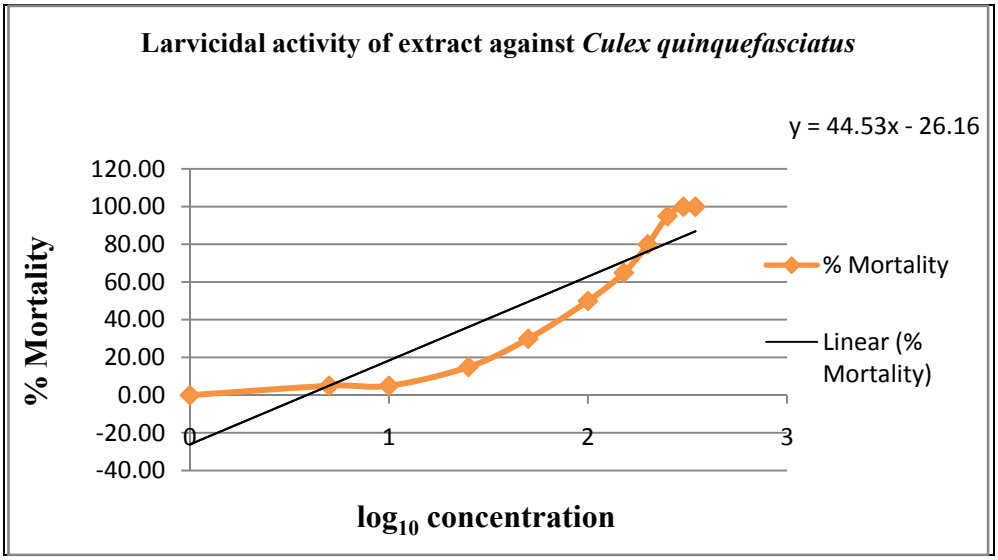
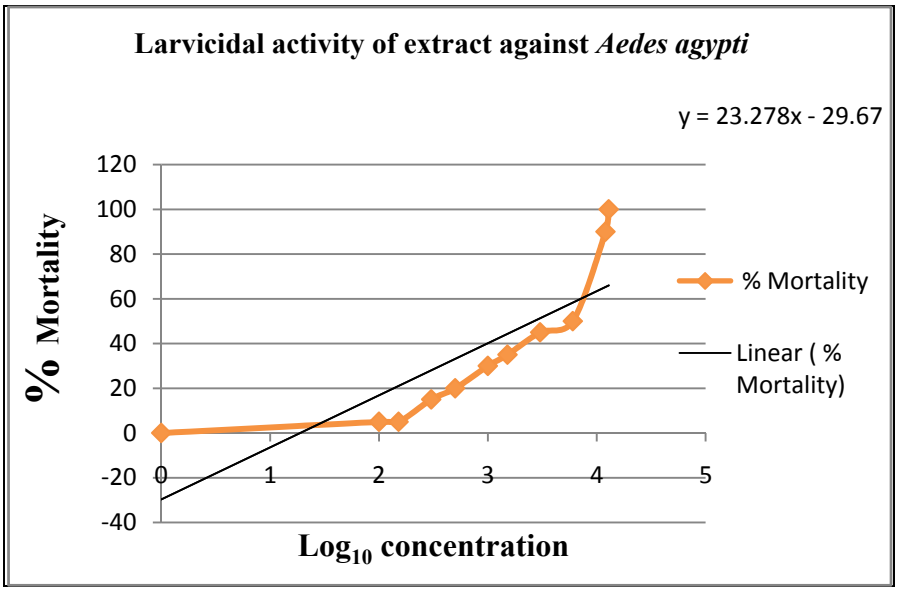


Table 20: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Aedes agypti*

S. No	Conc. (ppm)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Probit	Probit	Regression value
0	0	0	0	0	0	0	0	0
1	100	2.00	20	1.00	5.00	3.35	3.00	4.624
2	150	2.18	20	1.00	5.00	3.35	3.00	4.624
3	300	2.48	20	3.00	15.00	3.96	4.00	5.234
4	500	2.70	20	4.00	20.00	4.16	4.00	5.434
5	1000	3.00	20	6.00	30.00	4.48	4.00	5.754
6	1500	3.18	20	7.00	35.00	4.62	5.00	5.894
7	3000	3.48	20	9.00	45.00	4.87	5.00	6.144
8	6000	3.78	20	10.00	50.00	5	5.00	6.274
9	12000	4.08	20	18.00	90.00	6.28	6.00	7.554
10	13000	4.11	20	20.00	100.00			
LC50				**51.31ppm or 0.051%v/v**				
LC90				**405.88ppm or 0.405%v/v**				

Fig. 27: Larvicidal activity of methanolic extract of *Citrullus lanatus*against *Aedes agypti*



From the **Table 20** it was observed that a mortality of 100.00 ± 0.00 was observed for *Aedes agypti.* The LC_{50} and LC_{90} values were calculated using Probit analysis. The percentage mortality calculated using Abbott's formula versus log concentration was plotted and Y=50 is substituted in the resulting linear equation to obtain the X value. The linear regression equation was found to be y = 23.278x - 29.67 for activity against *Aedes agypti.* The antilog of X was then the LC_{50} (conc. of 50% mortality) or LC_{90} (conc. of 90% mortality) value.

The LC_{50} was found to be **2645ppm or 2.645%v/v** for *Aedes agypti*. The LC_{90} value was found to be **138326ppm or 138.326%v/v** for *Aedes agypti*. One hundred percent mortality was observed for the concentrations tested against the organisms. The extract was effective against *Aedes agypti.*

One hundred percent mortality was observed for various concentrations ranging for above organisms tested were observed within 24h of the start of the experiment.

In the present study, methanolic extract of *Citrullus lanatus* showed promising larvicidal activity against important vectors of malaria, filarial, dengue, dengue haemorrhagic fever, yellow fever, chikungunya.

Mosquito control programmes largely target the larval stage at their breeding sites with larvicides. Larviciding is a successful method of reducing mosquito population in their breeding place before they emerge as adults. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product –based mosquito abatement practices. Plant extracts are reported to be eco-friendly mosquito control agents.

Plants are well known to contain a complex of chemicals with bioactive potential like deterrents or attractants. Leaves are available throughout the year could be easily collected without any additional cost. Therefore, leaves extracts could be used as a larvicidal agent in an integrated vector control program.

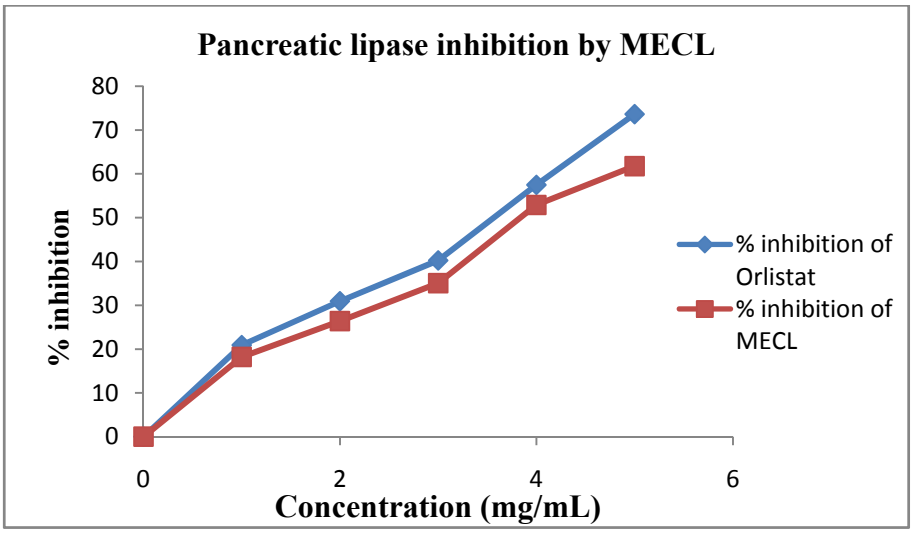
SECTION C - PANCREATIC LIPASE INHIBITION ASSAY

The results obtained for pancreatic lipase inhibition assay are presented in **Table 21** and the graphical representation is presented in **Fig.28.**

Table 21: Pancreatic lipase inhibition of MECL

S. No	Conc. in mg/ml	% inhibition of Orlistat	% inhibition of MECL
1	1.0	20.91 ± 0.293	18.20 ± 0.306
2	2.0	30.93 ± 0.583	26.40 ± 0.363
3	3.0	40.23 ± 0.666	35.04 ± 0.416
4	4.0	57.47 ± 0.490	52.90 ± 0.523
5	5.0	73.63 ± 0.553	61.77 ± 1.102
IC_{50}		**3.420 mg/mL**	**3.962 mg/mL**

Fig.28: Determination Pancreatic lipase inhibition of MECL



From the **table 21,** it can be seen that the methanolic extract of *Citrullus lanatus* showed a percentage inhibition 61.77 ± 1.102 at a concentration of 5.0mg/mL. The IC_{50} values calculated using the linear regression analysis was found to be 3.962mg/mL for methanolic extract of *Citrullus lanatus.* The Orlistat showed a percentage inhibition 73.63 ± 0.553 at a concentration of 5.0 mg/mL, IC_{50} value was found to be 3.420mg/mL. The extract of *Citrullus lanatus* possesses a good inhibitory activity on pancreatic lipase.

The phytochemical screening of methanolic extract of *Citrullus lanatus* showed the presence of flavanoid, tannins, terpenes, phenols and saponins. Results of the study indicate that methanolic extract of *Citrullus lanatus* inhibits pancreatic lipase. The inhibitory activities of different concentration of Standard and methanolic extract of *Citrullus lanatus* were tested aginst chicken pancreatic lipase using olive oil as the substrate.

The dose dependent pancreatic lipase inhibitory activity was observed. i.e inhibition of enzyme was increased on increasing concentration of extract. Polyphenols like flavanoids and tannins with an inhibitory effect on pancreatic lipase activity, which could be applied in the management of the obesity epidemic.

Overweight, obesity and weight change: Overweight (BMI ≥25 and < 30kg/m^2) or obese (BMI ≥ 30kg/m^2) individual have a higher risk for many types of cancer compared with individuals whose BMI is considered within the normal range (18.5 to < 25 kg/m^2). The cancers most consistently associated with overweight and obesity are breast, colon/rectum, endometrium, pancreas, adenocarcinoma of the esophagus, kidney, gallbladder, and liver.

Obesity may also increase risk of mortality from some cancers, such as prostate. A growing body of evidence suggests that weight gain is associated with an increased risk of some cancers, breast cancer in particular. Increases in body weight during adulthood largely reflect increases in adipose tissue rather than lean mass. The total body fat may be a better measure of the risk for cancer than BMI.

Studies over decades have consistently shown a strong association between obesity and both insulin resistance and type 2 diabetes incidence, with risk of diabetes and earlier age at onset directly linked to obesity severity. For type 2 diabetes as well as certain cancers, some studies suggest that waist circumference, waist –to-hip ratio, or direct measures of visceral adiposity with risk independently of BMI.

The case for a causal relationship between obesity and disease is strengthened by evidence that weight loss lowers disease risk. In the case of diabetes, numerous studies have shown that weight loss decreases diabetes incidence and restores euglycemia in a significant fraction of individuals with type 2 diabetes. In the randomised, prospective, multicenter Diabetes prevention program trial, an intensive lifestyle intervention of diet and physical activity was associated with a 58% reduction in diabetes incidence in high risk individuals, and weight loss accounted for most of the effect.

Several studies suggest that diets high in foods with a high glycemic index or load are associated with an increased risk of type 2 diabetes. However evidence of their associations with cancer risk is mixed.

SECTION D - *IN VITRO* ANTI CANCER ACTIVITY (BREAST CANCER ACTIVITY BY USING HUMAN BREAST CANCER CELL LINES)

The *iIn vitro* anti-cancer (breast cancer) activity of methanolic extract of *Citrullus lanatus (MECL)* is tabulated in **Table 22 & 23**

Table 22: Various concentration and Absorbance of the MECL

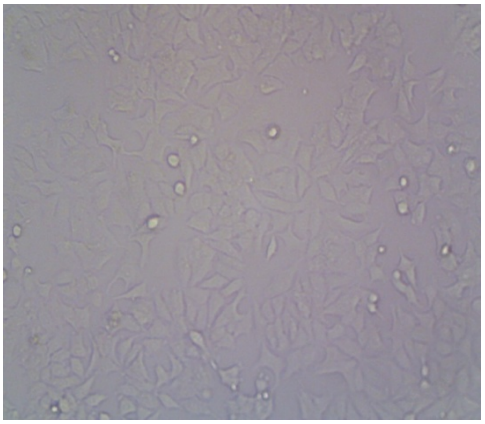
Conc	18.75 µg/mL	37.5 µg/mL	75 µg/mL	150 µg/mL	300 µg/mL	Control
ABS	0.316	0.313	0.299	0.293	0.277	0.305
	0.308	0.305	0.3	0.287	0.269	0.307
	0.31	0.292	0.292	0.285	0.279	0.309
Avg	**0.311**	**0.303**	**0.297**	**0.288**	**0.275**	**0.307**

Table 23: Percentage Cell viability of Methanolic extract of *Citrullus lanatus*.

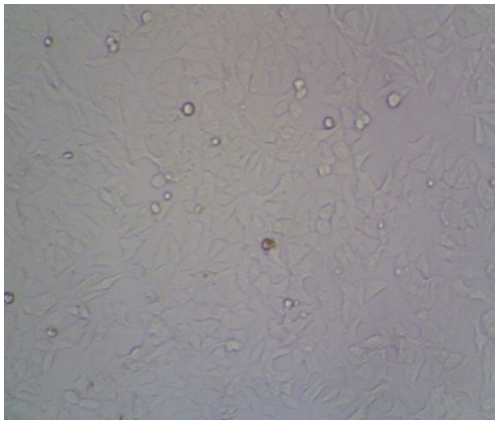
S. No	Conc. (µg/ml)	% Cell viability	% Inhibition
1	18.75	101.411	0.00
2	37.50	98.805	1.20
3	75.00	96.742	3.25
4	150.00	93.919	6.08
5	300.00	89.576	10.42

Fig.30: Photomicrograph of human breast cancer cell lines (MCF-7)

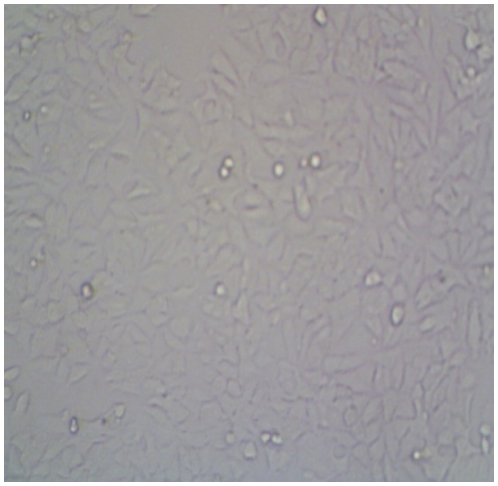
MECL at 18.75µg/mL



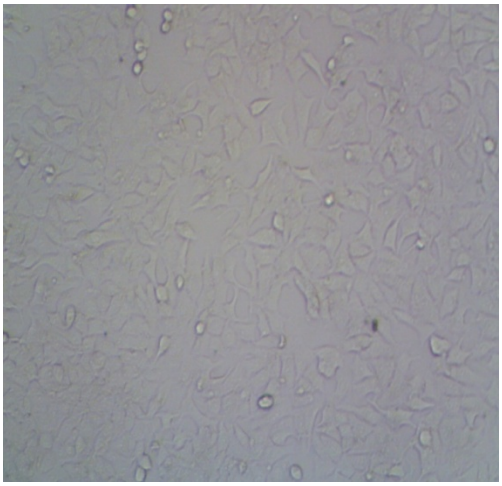
MECL at 37.50µg/mL



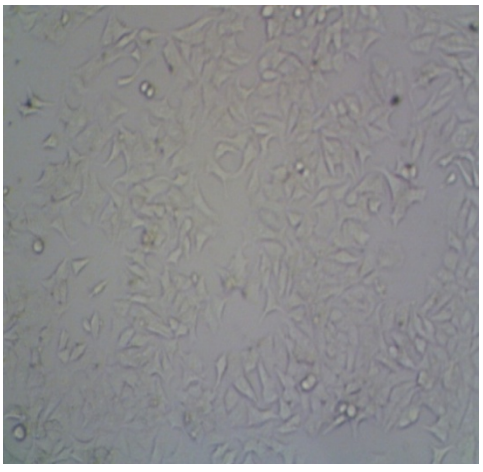
MECL at 75µg/mL



MECL at 150µg/mL



MECL at 300µg/mL



Control

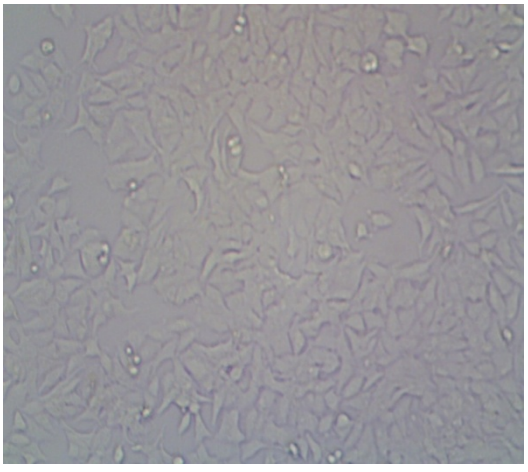
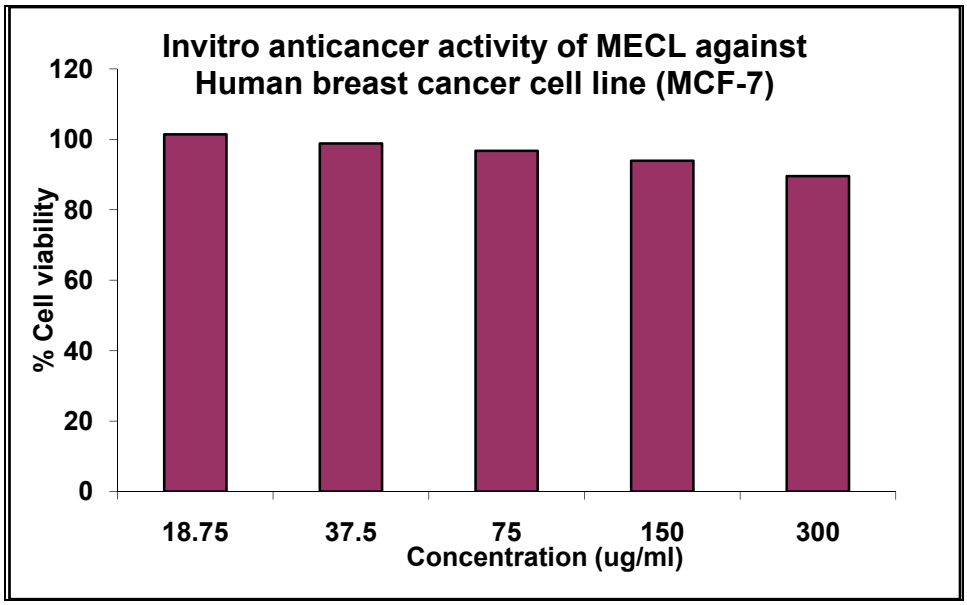


Fig. 29: Percentage Cell viability of methanolic extract of *Citrullus lanatus*.



The MECL showed a slight cytotoxic activity against human breast cancer cell lines MCF-7 at 300µg/mL with a cell viability of 89.57%. The percentage inhibition was found to be 10.42% at the concentration of 300µg/mL. The breast cancer activity was determined on human breast cancer cell lines MCF-7. The extract did not show a significant anticancer activity.

SECTION E - IN VITRO ANTIDIABETIC ACTIVITY

METHOD I : NON-ENZYMATIC GLYCOSYLATION OF HAEMOGLOBIN ASSAY

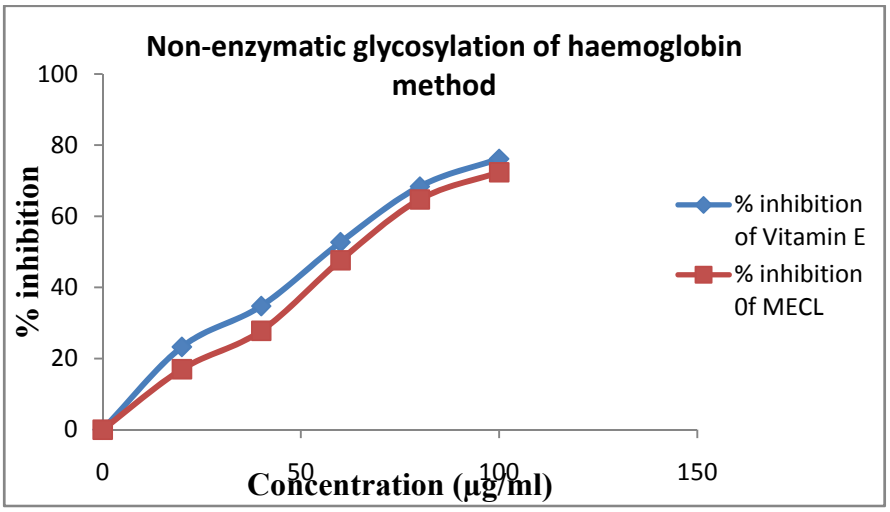
The results for *in vitro* non enzymatic glycosylation of haemoglobin assay are presented in **Table 24** and the graphical representation of the same in **Fig. 31.** The percentage inhibition was found to be 72.398 ± 0.221 and 76.171 ± 0.213 for MECL and α-tocopherol respectively. The IC_{50} value calculated using linear regression analysis was found to be **65.648 µg/mL** and **59.762 µg/mL** for MECL and α-tocopherol respectively.

Table 24: *In-vitro* Non-enzymatic glycosylation of haemoglobin method

S. No	Conc. in µg/ml	α-Tocopherol		Methanolic extract of *Citrullus lanatus*	
		Abs	% inhibition	Abs	% inhibition
1	20	0.159 ± 0.003	23.270 ± 1.674	0.147 ± 0.000	17.006 ± 0.496
2	40	0.187 ± 0.002	34.759 ± 1.016	0.169 ± 0.004	27.810 ± 1.847
3	60	0.258 ± 0.004	52.713 ± 0.813	0.233 ± 0.002	47.639 ± 0.589
4	80	0.386 ± 0.004	68.393 ± 0.342	0.346 ± 0.005	64.739 ± 0.623
5	100	0.512 ± 0.004	76.171 ± 0.213	0.442 ± 0.003	72.398 ± 0.221
IC_{50} Value		**59.762 µg/mL**		**65.648 µg/mL**	

*** mean of three readings ±SEM**

Fig.33 : *In-vitro* Non-enzymatic glycosylation of haemoglobin method



Plant extracts play an important role the inhibition of the glycosylation end products. An increase in the glycosylation was observed on incubation of hemoglobin with the increasing concentration of the glucose over a period of 72hrs. However, the plant extracts significantly inhibited the haemoglobin glycosylation which is indicated by the presence of increasing concentration of haemoglobin. *Citrullus lanatus* exhibited significant inhibition of glycosylation as compared with the standard drug α-tocopherol. The plant extracts also displayed the inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72hrs, indicating that the plant extracts

decreases the formation of the glucose- haemoglobin complex and thus amount of free haemoglobin increases.

METHOD II: IN-VITRO GLUCOSE UPTAKE IN YEAST CELLS

The rate of glucose transport across cell membrane in yeast cells system is presented in **Figs. 34 & 35, tables 25 & 26**.

Table 25: Percentage inhibition of Glucose uptake in 5mM glucose concentrations

S. No	Conc. µg/ml	Acarbose		Methanolic extract of *Citrullus lanatus*	
		Abs	% inhibition	Abs	% inhibition
1	40	0.102 ± 0.001	60.78 ± 0.220	0.099 ± 0.002	59.60 ± 0.386
2	80	0.117 ± 0.000	65.81 ± 0.222	0.106 ±0.002	62.26 ± 0.030
3	120	0.132 ± 0.001	69.70 ± 0.271	0.124 ± 0.003	67.74 ± 0.204
4	160	0.150 ± 0.002	73.33 ± 0.101	0.140 ± 0.001	71.43 ± 0.177
5	200	0.188 ±0.002	78.72 ± 0.248	0.152 ± 0.006	73.68 ± 0.362
IC_{50} Value		**74.083 µg/mL**		**80.218 µg/mL**	

*** mean of three readings ±SEM**

Fig.34 : % inhibition of Glucose uptake at 5mM concentration

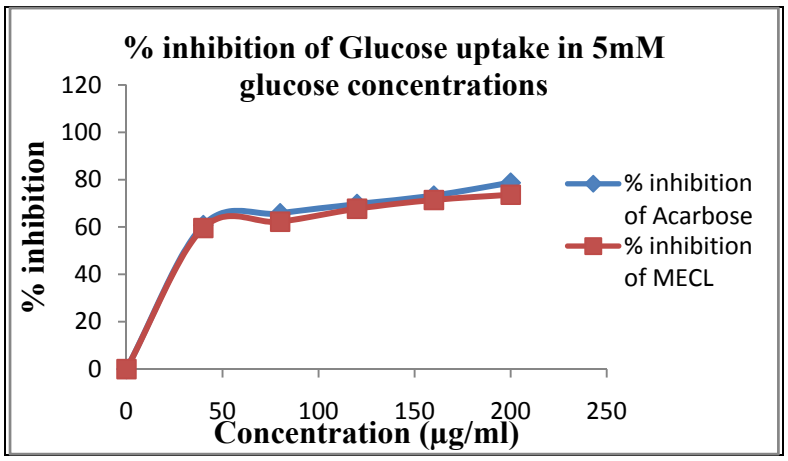


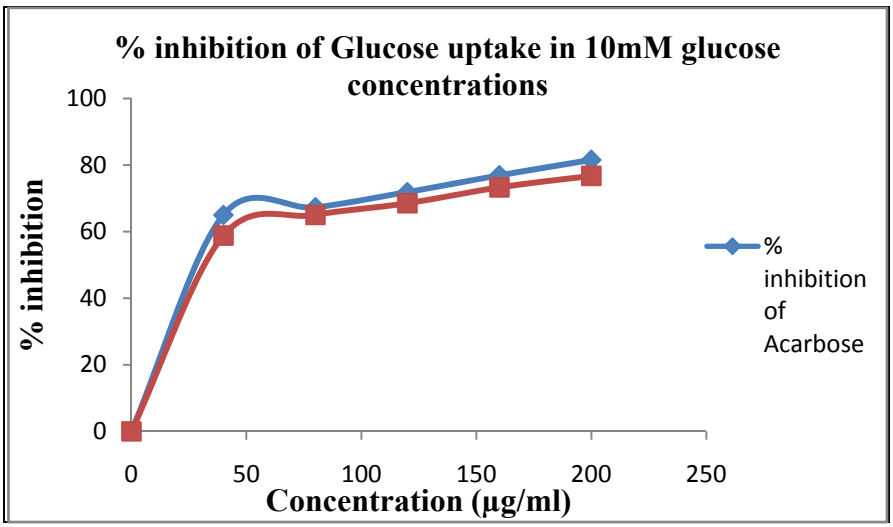
Table 26: Percentage inhibition of Glucose uptake at 10mM concentration

S. No	Conc. µg/ml	Acarbose		Methanolic extract of *Citrullus lanatus*	
		Absorbance	% inhibition	Absorbance	% inhibition
1	40	0.200 ± 0.003	65.00 ± 0.242	0.170 ± 0.004	58.82 ± 0.257
2	80	0.214 ± 0.002	67.28 ± 0.163	0.220 ± 0.001	65.00 ± 0.282
3	120	0.249 ± 0.004	71.88 ± 0.178	0.248 ± 0.001	68.54 ± 0.081
4	160	0.303 ± 0.003	76.89 ± 0.206	0.311 ± 0.003	73.28 ± 0.077
5	200	0.379 ± 0.001	81.53 ± 0.181	0.335 ± 0.003	76.74 ± 0.214
IC_{50} Value		**67.408 µg/mL**		**77.031 µg/mL**	

*** mean of three readings ±SEM**

The percentage inhibition was found to be 76.74 ± 0.214 and 81.53 ± 0.181 for MECL and acarbose at 200µg/mL respectively. The IC_{50} value calculated using linear regression analysis was found to be **77.031µg/mL** and **67.408 µg/mL** for MECL and acarbose respectively

Fig. 35: % inhibition of Glucose uptake in 10mM glucose concentrations



Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species. The i*n vitro*

assays of the present study indicated the methanolic extract of *Citrullus lanatus* possess good anti diabetic activity. In yeast, glucose transport takes place through facilitated diffusion. Type 2 Diabetes is characterised by the deficiency of insulin causing increased amount of glucose in blood. After the treatment of the yeast cells with these leaf extracts, the glucose uptake was found to increase in a dose dependent manner. The **Figs.32 & 33** depict the % increase in glucose uptake by the yeast cell at different glucose concentrations i.e.5mM and 10mM respectively. The methanolic extract of *Citrullus lanatus* exhibited significantly higher activity at all glucose concentrations showing the maximum increase in 10mM Glucose concentration. Results also indicated that *Citrullus lanatus* had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug Acarbose.

METHOD III -IN-VITRO ALPHA AMYLASE INHIBITION METHOD

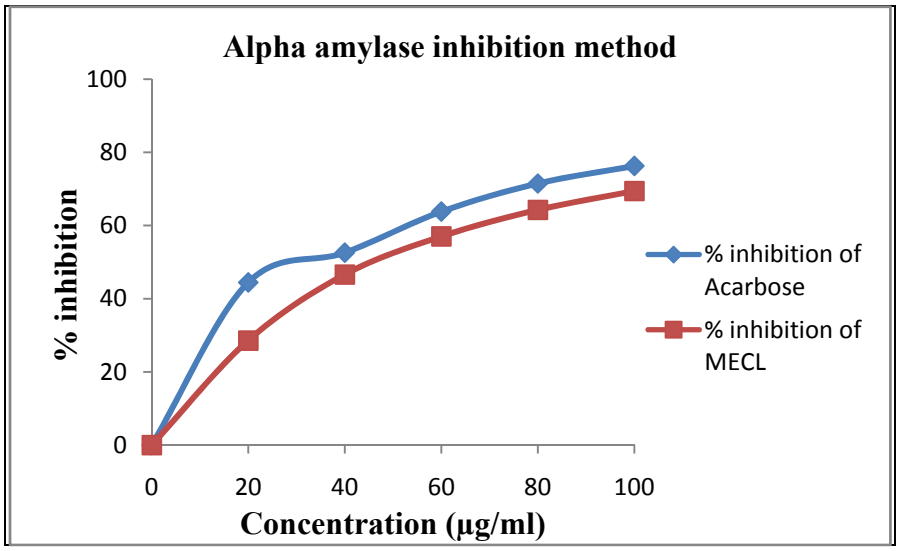
The α-amylase inhibitor effectiveness of methanolic extracts of the *Citrullus lanatus* was compared with Acarbose reference standard on the basis of their resulting IC_{50} values inhibited the activity of α-amylase with an IC_{50} of methanolic extract of *Citrullus lanatus was* 47.880 µg/mL. Acarbose, the positive control used in this study, inhibited the activity of α-amylase with an IC_{50} value estimated at 58.558µg/mL. The values are presented in **Table 27 and Fig. 34.**

Table 27: *In-vitro* Alpha amylase inhibition

S. No	Conc. µg/ml	Acarbose		Methanolic extract of *Citrullus lanatus*	
		Absorbance	% inhibition	Absorbance	% inhibition
1	20	0.099 ± 0.001	44.44 ± 0.646	0.077 ± 0.003	28.57 ± 3.185
2	40	0.116 ± 0.004	52.58 ± 1.751	0.103 ± 0.002	46.60 ± 1.020
3	60	0.152 ± 0.003	63.81 ± 0.844	0.128 ± 0.004	57.03 ± 1.341
4	80	0.193 ± 0.002	71.50 ± 0.340	0.154 ± 0.003	64.28 ± 0.768
5	100	0.232 ± 0.006	76.29 ± 0.658	0.186 ± 0.002	69.44 ± 0.410
IC_{50} Value		**47.880 µg/mL**		**58.558 µg/mL**	

*** mean of three readings ±SEM**

Fig. 36: In-vitro Alpha amylase inhibition method



The percentage inhibition was found to be 69.44 ± 0.410and 76.29 ± 0.658 for MECL and acarbose at 100µg/mL respectively. The IC_{50} value calculated using linear regression analysis was found to be **58.558 µg/mL** and **47.880 µg/mL** for MECL and acarbose respectively

Alpha amylase is an enzyme that hydrolyses alphabonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in mono and disaccharide. As the result shows methanolic extract of *Citrullus lanatus* significant activity as compared to acarbose standard drug.

Drugs that inhibit carbohydrate hydrolyzing enzymes have been demonstrated to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting insulin secretion of non insulin dependent diabetic patients. The results of *in vitro* studies showed that *Citrullus lanatus* inhibits α-amylase activity. Natural health products of vegetable origin were clearly indicated as a promising avenue for the prevention of chronic diseases (Punitha & Manoharan 2006).

METHOD IV - *IN-VITRO* ALPHA GLUCOSIDASE INHIBITION METHOD

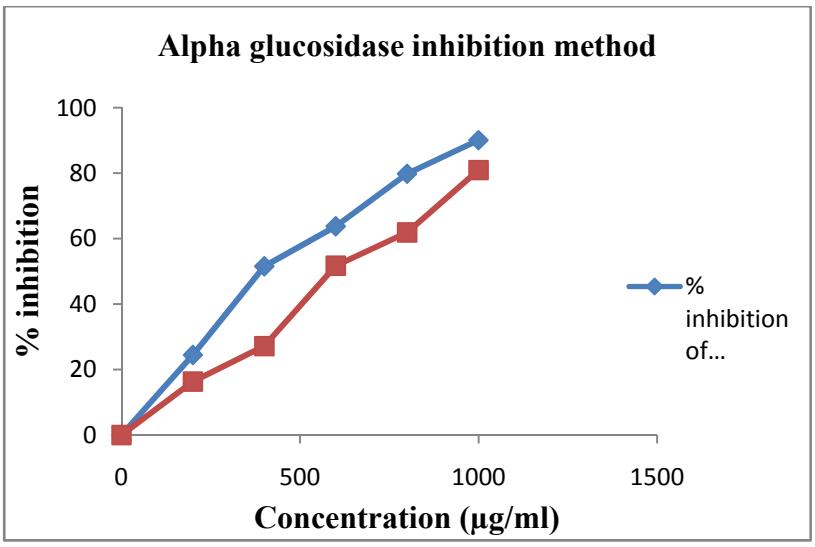
The values for in vitro alpha glucosidase inhibition are presented in **Table 28 and Fig.35.**

Table 28: *In-vitro* α-glucosidase inhibition assay

S. No	Conc. µg/ml	Acarbose		Methanolic extract of *Citrullus lanatus*	
		Absorbance	% inhibition	Absorbance	% inhibition
1	200	0.809 ± 0.004	24.46 ± 0.457	0.896 ± 0.002	16.34 ± 0.189
2	400	0.519 ± 0.002	51.54 ± 0.188	0.780 ± 0.001	27.17 ± 0.136
3	600	0.388 ± 0.001	63.77 ± 0.113	0.517 ± 0.002	51.73 ± 0.270
4	800	0.216 ± 0.001	79.83 ± 0.136	0.408 ± 0.002	61.90 ± 0.188
5	1000	0.106 ± 0/002	90.10 ± 0.243	0.204 ± 0.002	80.95 ± 0.273
IC_{50} Value		482.188 µg/mL		627.270 µg/mL	

* **mean of three readings ±SEM**

Fig. 37 : *In-vitro* α-glucosidase inhibition assay



The α-glucosidase inhibitor effectiveness of methanolic extracts of the *Citrullus lanatus* was compared with acarbose reference standard on the basis of their resulting IC_{50} values inhibited the activity of α-glucosidase with an IC_{50} of methanolic extract of *Citrullus*

lanatus was 627.270µg/mL. Acarbose, the positive control used in this study, inhibited the activity of α-glucosidase with an IC_{50} value estimated at 482.188µg/mL.

In *Diabetes mellitus*, control of postprandial plasma glucose level is critical in the early treatment. An inhibition of enzymes involved in the metabolism of carbohydrate is one of the therapeutic approaches for reducing postprandial hyperglycemia.

α-glucosidase is a key enzyme in carbohydrate digestion. It catalyzes the hydrolysis of 1,4-α-glucosidic bonds within carbohydrates with release of α-glucose and promotes the increase of blood glucose level after meal. α-glucosidase inhibitors antagonize the activity of α-glucosidase, thereby delaying intestinal carbohydrate absorption and slowing the sharp rise in blood sugar levels that diabetic patients typically experience after meals. For this reason, α-glucosidase inhibitors, such as acarbose and voglibose, are clinically used as oral antihyperglycemic agents. However, they often cause severe gastrointestinal side effects. Therefore, search for new α-glucosidase inhibitors from natural resources has become an attractive approach for the treatment of postprandial hyperglycemia.

In the present study, methanolic extract of *Citrullus lanatus* was screened for their alpha-glucosidase inhibitory potential. The extract showed better alpha-glucosidase inhibition property. Alpha-glucosidase inhibitors have a potential for the treatment of diabetes because they reduce diet-induced hyperglycemia.

The extract possessed a significant *in vitro* antidiabetic acitivity and hence *in vivo* studies are further required for providing a scientific information on the plant.

SUMMARY AND CONCLUSION

CHAPTER VIII

SUMMARY AND CONCLUSION

The present study entitled the **"Pharmacognostic, Phytochemical and Pharmacological evaluation of the Leaves of *Citrullus lanatus* (Thunb.) Matsum. & Nakai. (Cucurbitaceae)"** focuses on a plant which is commonly available throughout India and traditionally used in treatment of various ailments.

Studies on the leaves of *Citrullus lanatus* are still lacking. Hence to exploit its potential use prompted the present study to investigate the leaves of this plant with clear scientific protocol.

The chapter on **Literature Review deals** with the information regarding the pharmacognostical, phytochemical and pharmacological evaluation of the *Citrullus lanatus* plant and other species of Citrullus.

The chapter on **Pharmacognostical studies** highlights on

- Macroscopical features were studied and the adherence of general characters to the family *Citrullus lanatus* was found.
- Microscopical study reveals the presence of actinocytic stomata, multi cellular uniseriate unbranched epidermal trichomes. Vascular system of the midribis multistranded, a large abaxial median bundle, two adaxial bundle. All the bundles are bicollateral having phloem strand both outer and inner side of the xylem. The epidermal cells are small elliptical or rectangular and thin walled. Spongy parenchyma cells small and spherical. Palisade zone consist of single layer of cylindrical cells, loosely arranged.

- Quantitative microscopical studies namely stomatal number, stomatal index, vein islet number, vein termination number, ash value, extractive value, loss on drying value etc.,
- Also studied cell powder microscopy, fluorescence analysis of powder and the results helps in achieving a trouble-free identification and authenticity of the plant leaf or in powder form in future.

The chapter on **Phytochemical Evaluation** deals with

- Preliminary phytochemical screening reveals the presence of carbohydrate, alkaloids, flavanoids, protein & aminoacids, glycosides etc.,
- Quantitative determination of secondary metabolites (phenol, flavanoid, tannin content) has been carried out.
- TLC & HPTLC studies showed the presence of flavanoid, phenolic compound and tannin. In HPTLC studies the extract was compared with standards and the presence of quercetin, gallic aci and catechin were formed.
- The vitamin B_1, B_2 and vitamin C were estimated.

The chapter on **Pharmacological studies** focuses

- **The Antioxidant activity** by various methods and the extract possessed a good antioxidant property due to the presence of Vitamin-C, poly phenolic, flavonoid content.

- The **larvicidal effect** of extract of the *Citrullus lanatus* was carried out by standard procedure. In the present study, methanolic extract of citrullus lanatus showed promising larvicidal activity aginst important vectors of malaria, filariasis, dengue, dengue haemorrhagic fever, yellow fever.

- Obesity can lead to variety serious diseases, including hypertension, hyperlipidemia, atherosclerosis, and type II diabetes. The pancreatic lipase inhibitors of digestive lipases reduce dietary fat absorption and hence act as anti-obesity agents. The *Citrullus lanatus* extract have a capacity to inhibit the pancreatic lipase.

- The extract showed **anti cancer (breast cancer)** activity aginst human breast cancer cell lines (MCF-7) which was evaluated by MTT assay. The risk of breast cancer increases gradually as a woman gets older. The high concentration of the MECL used for inhibiting human breast cancer cell lines (MCF-7). Type 2 diabetes and breast cancer share many risk factors. The extract *Citrullus lanatus* showed high antidiabetic activity with high concentration showed breast cancer activity.

- The **antidiabetic activity** of extract of the leaves of *Citrullus lanatus* was carried out by Non-enzymatic glycosylation of haemoglobin Assay method, Glucose uptake in yeast cells method (% inhibition of Glucose uptake in 5mM glucose concentrations & % inhibition of Glucose uptake in 10mM glucose concentrations) Alpha amylase inhibition assay method & Alpha glucosidase inhibition assay method. The extract showed better alpha-glucosidase inhibition property.

The extract exhibit significant inhibition of glycosylation as compared with the standard drug alpha tocopherol. Decreases the formation of the glucose- haemoglobin complex and thus amount of free haemoglobin increases.

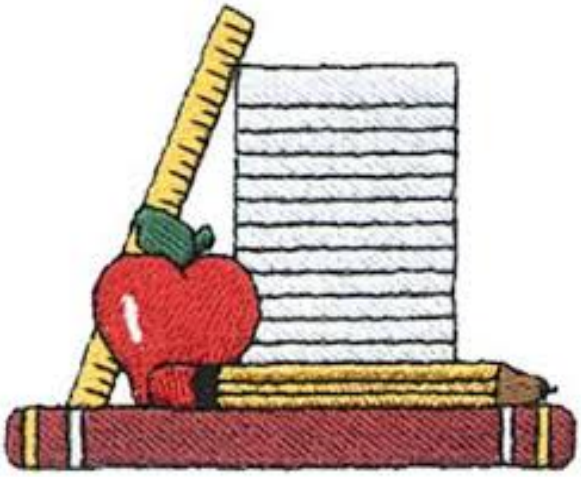
The extract showed greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug Acarbose. Type 2 Diabetes is characterised by the deficiency of insulin causing increased amount of glucose in blood. After the treatment of the yeast cells with these leaf extracts, the glucose uptake was found to increase in a dose dependent manner.

Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in mono and disaccharide. The result showed extract of *Citrullus lanatus* significant activity as compared to acarbose standard drug.

Alpha-glucosidase inhibitors have a potential for the treatment of diabetes because they reduce diet-induced hyperglycemia. The extract showed better alpha-glucosidase inhibition property.

The i*n vitro* assays of the present study indicated the methanolic extract of *Citrullus lanatus* possess good anti diabetic activity.

- *Citrallus lanatus* (water melon) is popular in indigenous system of folk medicine. The leaf extract of *Citrullus lanatus* contain bioactive compounds such as flavanoid, phenolic compound, tannin, triterpenes, sterols and alkaloids, vitamins. The extract may serve as a lead medicinal plant to synthesise various semi-synthetic drugs to treat various life threatening disease.



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